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Diverse Mutations in the Gene for Cartilage Oligomeric Matrix Protein in the Pseudoachondroplasia–Multiple Epiphyseal Dysplasia Disease Spectrum

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Summary

Pseudoachondroplasia (PSACH) and multiple epiphyseal dysplasia (MED) are autosomal dominant osteochondrodysplasias that result in mild to severe short-limb dwarfism and early-onset osteoarthrosis. PSACH and some forms of MED result from mutations in the gene for cartilage oligomeric matrix protein (COMP; OMIM 600310 [http://www3.ncbi.nlm.nih.gov:80/htbin-post/Omim/dispmim?600310]). We report the identification of COMP mutations in an additional 14 families with PSACH or MED phenotypes. Mutations predicted to result in single–amino acid deletions or substitutions, all in the region of the COMP gene encoding the calmodulin-like repeat elements, were identified in patients with moderate to severe PSACH. We also identified within this domain a missense mutation that produced MED Fairbank. In two families, one with mild PSACH and the second with a form of MED, we identified different substitutions for a residue in the carboxyl-terminal globular region of COMP. Both the clinical presentations of these two families and the identification of COMP-gene mutations provide evidence of phenotypic overlap between PSACH and MED. These data also reveal a role for the carboxyl-terminal domain in the structure and/or function of COMP.

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Introduction

Pseudoachondroplasia (PSACH; OMIM 177170 [http://www3.ncbi.nlm.nih.gov:80/htbin-post/Omim/dispmim?177170]) and multiple epiphyseal dysplasia (MED; OMIM 132400 [http://www3.ncbi.nlm.nih.gov:80/htbin-post/Omim/dispmim?132400]) are autosomal dominant osteochondrodysplasias that result in mild to severe short-limb dwarfism and early-onset osteoarthrosis (International Working Group on Constitutional Diseases of Bone 1992). PSACH is the clinically more severe phenotype, characterized by marked short stature, short fingers, loose joints with ligamentous laxity, deformity of the legs, and scoliosis. Radiographic features include small irregular epiphyses with delayed ossification, irregular metaphyses, and delayed ossification of the annular epiphyses of the vertebrae, resulting in a characteristic anterior beaking of the vertebral bodies that is apparent on lateral projection. Milder cases of PSACH exhibit similar radiographic features, but short stature is less pronounced and there is less deformity than in more severe cases (Maroteaux et al. 1980; Rimoin et al. 1994). MED, which is divided into the more severe Fairbank type (Fairbank 1947) and the milder Ribbing type (Ribbing 1937), is less severe than PSACH. Patients with MED exhibit normal stature, or mild short stature, and early-onset osteoarthrosis that becomes apparent in mid- to late childhood. Radiographically, the epiphyses are irregular and show poor ossification, but metaphyseal abnormalities are mild or absent. The spine is typically radiographically normal. Morphological studies of cartilage from PSACH and MED patients show accumulation of material in the rough endoplasmic reticulum (RER) of chondrocytes (Maynard et al. 1972; Stanescu et al. 1977, 1982, 1993; Rimoin et al. 1994). In most PSACH and in some MED cases, the accumulated material forms a unique lamellar structure (Maynard et al. 1972; Stanescu et al. 1977, 1982, 1993).

Initially, the mild and severe PSACH phenotypes were linked to markers in the pericentromeric region of chromosome 19 (Briggs et al. 1993; Hecht et al. 1993), and they were subsequently found to result from mutations in the gene for cartilage oligomeric matrix protein (COMP) (Briggs et al. 1995*a;* Hecht et al. 1995). Some forms of MED were also linked to this region (Oehlmann et al. 1994), and COMP mutations were identified in patients with the Fairbank (Briggs et al. 1995*a*) and Ribbing (Ballo et al. 1997) types of MED. MED Fairbank can also result from defects in the $\alpha 2$ chain of type IX collagen (OMIM 600204 [http://www3.ncbi.nlm.nih.gov:80/htbin-post/Omim/dispmim?600204]) (Briggs et al. 1994; Muragaki et al. 1996) and from defects in at least one additional gene (OMIM 600969 [http://www3.ncbi.nlm.nih.gov:80/htbin-post/Omim/dispmim?600969]) (Deere et al. 1995).

COMP is a 524-kD homopentameric glycoprotein that is expressed prominently in the territorial matrix surrounding chondrocytes (Hedbom et al. 1992). It is a member of the thrombospondin gene family and is also referred to as "thrombospondin 5." COMP is a modular protein containing an amino-terminal domain, four epidermal growth factor–like repeats, eight calmodulin-like (CaM-like) repeats, and a large carboxyl-terminal globular domain (Oldberg et al. 1992). To date, all of the reported COMP-gene mutations that result in PSACH or MED phenotypes have been in the region of the gene encoding the CaM-like domain (Briggs et al. 1995*a;* Hecht et al. 1995; Ballo et al. 1997; Loughlin et al. [in press]). These have included point mutations that result in single–amino acid substitutions for conserved residues and in-frame deletions that delete codon(s) for one or more residues.

We report the identification of mutations in an additional 14 families with phenotypes within the PSACH-MED disease spectrum. Twelve mutations were identified in the CaM-like domain; this finding confirms the importance of this region in the structure and/or function of COMP. We have also identified two mutations in the carboxyl-terminal domain of COMP, thereby extending the range of COMP-gene mutations that can produce PSACH or MED phenotypes. These data provide evidence that the carboxyl terminal domain of COMP has an important structural and/or functional role in the cartilage extracellular matrix.

Patients and Methods

Clinical Summary

The clinical and radiographic features of each patient or family were reviewed by at least two clinical geneticists and/or radiologists. COMP mutations were identified in eight affected individuals who were sporadic products of nonconsanguinous unions (International Skeletal Dysplasia Registry reference numbers R91-064, R92-207, R95-108, R95-109, and R95-110, and University of Gent patients BW, CM, and WL). The proband in family R95-107 was the offspring of an affected father and an unaffected mother. All affected individuals in these families exhibited the typical clinical features of PSACH: short-limb dwarfism, small hands with short fingers, loose joints, a waddling gait, and varum and/or valgum deformity of the lower limbs. Childhood radiographs of all the patients were reviewed; they demonstrated small, irregular epiphyses in the hands and knees and irregular metaphyses. The hips exhibited microepiphyses and acetabular irregularities. Anterior beaking of the vertebral bodies was apparent on lateral projection. These features are diagnostic for typical PSACH.

The pedigrees of the other five families in which COMP mutations were identified are shown in figure 1. The clinical features of these families are described below.

Family R94-344 (*MED Fairbank*).—The proband, III-1 (fig. 1), was first seen at 15 years of age. She was short in stature (height 144 cm), and she complained of pain in the knees. Her hands were normal. Radiographs showed normal hands and hips, but the tibial epiphyses were irregular, with a squared aspect. Her affected sister, III-3, was also short in stature and had involvement of the knees and hips. The femoral head was small and irregular, but the severity of the deformities was somewhat less striking than the deformities in other cases of MED Fairbank. The affected father (II-4) of the proband had knee involvement, but the hips were unaffected. The affected grandmother (I-2) had severe hip dysplasia that required surgical replacement of the femoral head.

Family R83-130 (*mild PSACH*).—Affected individuals were identified initially on the basis of their waddling gaits. In childhood, they developed chronic hip pain that was exacerbated by moderate exercise. Heights of the affected individuals ranged from 145 cm (III-6, fig. 1) to 180 cm (IV-2). Radiographs showed significant epiphyseal and metaphyseal changes in the joints. The hips showed microepiphyses and irregular acetabulae. The spines showed platyspondyly and anterior beaking of the vertebrae.

Family R92-002 (*unclassified MED*).—The earliest symptoms included a waddling gait and lumbar lordosis. The height of the only affected adult in the family (II-2, fig. 1) was 147 cm. Radiographs showed small, irregular epiphyses, particularly at the proximal femur, and mild metaphyseal abnormalities. The hands were atypical of PSACH or MED in that they were short, in all segments, with irregular metaphyses and large epiphyses. In childhood, neither anterior beaking of the vertebrae nor platyspondyly was apparent. The phenotype

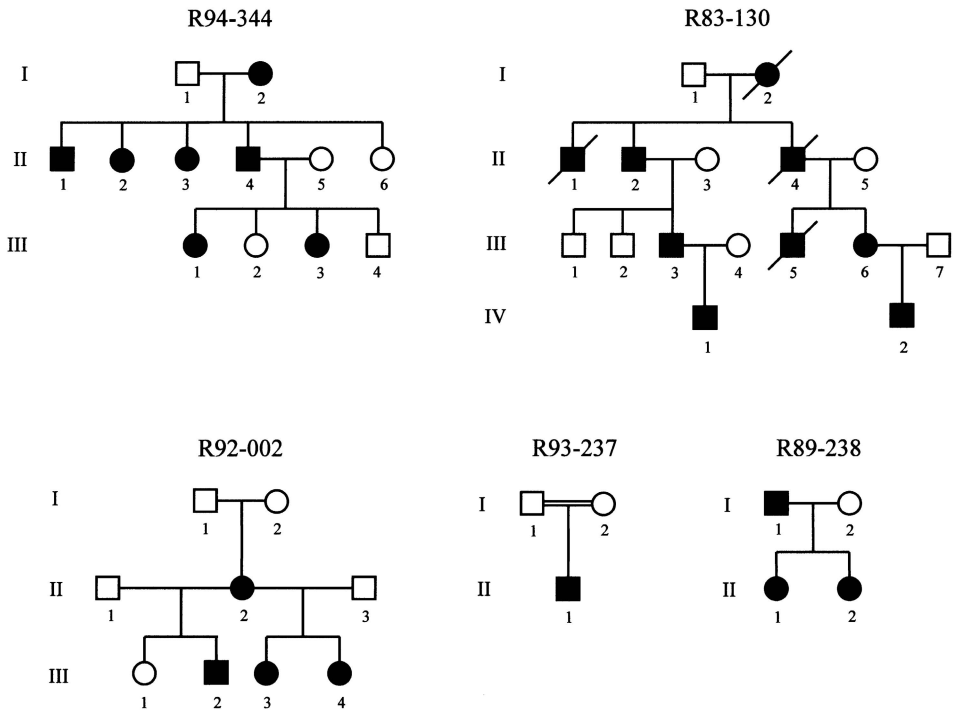

Figure 1 Pedigrees of five families in which COMP mutations were identified. The International Skeletal Dysplasia Registry reference number is shown above each pedigree. Blackened symbols indicate affected individuals. Generations are indicated by roman numerals to the left of each pedigree. Individuals within a generation are indicated by arabic numerals.

of the family is most consistent with a diagnosis of MED, but it has some features of PSACH. However, in some respects, it resembles neither PSACH nor MED.

Family R93-237 (*typical PSACH*).—The male proband was the first offspring of unaffected first-cousin parents of Pakistani origin. Results of a prenatal fetal ultrasound were normal, and the boy was normal at birth, with a birth length at the 50th percentile. By 27 mo of age, his height had fallen below the 5th percentile. At 6 years of age, he was 91 cm tall (3d percentile), and he had mild bowing of the lower extremities and some limitation of movement at the elbows and knees. Radiographs showed platyspondyly, with interpedicular narrowing in the lumbar vertebrae, delayed ossification of the epiphyses of the long bones, widened metaphyses, and a flattened acetabular roof. There was generalized brachydactyly of the hands, with hypoplastic epiphyses and cupped metaphyses.

Family R89-238 (*typical PSACH*).—The phenotype in this family is characterized by marked short stature, a waddling gait, pes varum, joint hyperlaxity, and progressive deformity at the knees, resulting in a "windswept" appearance. Mild lordosis and scoliosis were noted. At 6 years of age, the older sister (II-1, fig. 1) underwent surgical correction of the knee deformity, but by 12 years of age, medial deformity of the knees recurred. At 12 years of age, she was 119 cm tall (mean height of a 6-year-old child) and had short, stubby hands. Her affected younger sister (II-2) had a similar phenotype and was 101 cm tall at 6.5 years of age (>3 SD below the mean). Radiographs of both children showed microepiphyses and irregular metaphyses of the hips, and the vertebral bodies showed anterior beaking, diagnostic for PSACH.

PCR Amplification and SSCP Analysis

PCR amplifications were performed in 100-μl reactions, containing 1.5 mM $MgCl_2$, 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 200 μM each dNTP, 10 pmol each primer, 50–100 ng genomic DNA, and 1 U *Taq* DNA polymerase (Perkin-Elmer). Cycling conditions consisted of an initial 3 min at 94°C, followed by 35 cycles of 1 min at 94°C, 1 min at 55°C, and 1 min at 70°C, with a final incubation of 5 min at 70°C. PCR products were resolved by electrophoresis through nondenaturing 6% polyacrylamide gels (37.5 acrylamide:1 bis) in 1 × Tris-Borate-EDTA buffer. COMP exons 10–19 were analyzed. Oligonucleotide sequences are given in table 1. For SSCP analysis, 2 μCi α-[^{33}P]dCTP was included in the PCR reaction, and the products were resolved on Hydrolink mutation detection electrophoresis gels (AT

Table 1

Exons Amplified, Primer Names and Sequences, and Sizes of Amplified Products

Exon(s)	Primer Names	Primer Sequences	Product Size (bp)
10	I9F1	5′-TGA GGA GTG TGA CCT TTG CC-3′	279
	I10R1	5′-AGC CGA ATC CCG CCT TCG GTG-3′	
11	I10F1	5′-CTT GGG CTC TGG TCC CGT GG-3′	181
	I11R1	5′-GCT TAC CCA GCT GGA GTC TG-3′	
12	I11F2	5′-ATT TCC TCT GTC TGA TTA TGG-3′	168
	I12R1	5′-CCA GAG ACA ATG AGC TCT CCAG-3′	
13	I12F2	5′-GGG TAG CCT TTG ACA AAA CG-3′	223
	1492R	5′-GTT AGG CAC CAG GCG GCA G-3′	
14	I13F1	5′-TGA CTT TAG CCC ACC GAG GG-3′	281
	I14R1	5′-CTC AGC ATA GGC CTC ACT GTG-3′	
15	I14F1	5′-CAC AGT GAG GCC TAT GCT GA-3′	164
	I15R2	5′-GTG GCA GGA TAG CGC TGC TC-3′	
16	I15F1	5′-GCG TTC GGA AAG GCC ACT GC-3′	319
	I16R1	5′-CTA AGT GGC TGT AAA GGG TTT-3′	
17	I16F1	5′-GCC CAC CGA GGT CTC TGA CC-3′	275
	I17R1	5′-GGC ACT CCC ACC TGG GCC TG-3′	
18/19	2116F	5′-GCG ATT CTA TGA GGG CCC TGA-3′	319
	2342R	5′-GCG GTG AGG GTG GCT GTC AT-3′	

Biochem), according to the manufacturer's protocol, except that the gels were precooled and run for 2–3 h at 4°C, at 50 W.

DNA Sequence Analysis

PCR products were cleaned by use of the QIAquick kit (Qiagen) and were ligated into the pCRII TA cloning vector (Invitrogen). DNA was isolated from the clones, and sequences were determined by use of Sequenase and reagents from a kit (US Biochemical). Direct sequence analysis of PCR products was determined by means of dye terminator chemistry followed by resolution on the ABI PRISM 377 automated sequencer (Perkin-Elmer).

Results

The patient panel included 20 families with typical PSACH, 2 families with mild PSACH, 6 families with MED Fairbank, 3 families with MED Ribbing, and 13 families with unclassified forms of MED. By means of heteroduplex, SSCP, and DNA sequence analysis, we identified COMP-gene mutations in 14 individuals or families who had phenotypes in the PSACH-MED clinical spectrum. The phenotypes are detailed in the clinical summary, the relevant pedigrees are shown in fig. 1, and the mutations are summarized in table 2.

All individuals affected with typical PSACH, including BW, WL, and members of families R89-238, R91-064, R92-207, R95-108, and R95-110, were heterozygous for a 3-bp deletion identified previously in patients with this phenotype (Hecht et al. 1995). The deletion removed one of five consecutive aspartic acid (GAC) codons corresponding to residues 469–473 of the protein, within the seventh CaM-like repeat. The repeated nature of the sequence (C GAC GAC GAC GAC GAC) does not allow precise determination of the codon that is deleted in these patients. We hypothesize that this repeat unit predisposes to mutations of this type, which may occur via slipped base pairing during DNA replication (Kunkel 1993; Ashley and Warren 1996). Ultrastructural studies of cartilage from an individual in family R89-238 showed the typical accumulation of material with a lamellar appearance in the RER of chondrocytes that is common in cases of PSACH (Cohn et al. 1996). Although chondrocytes from R95-110 showed RER inclusions with a smooth to grainy (rather than lamellar) appearance (data not shown), cell preservation was poor, so few cells were examined.

A similar but shorter repeated sequence, C GAC GAC GAC, is present in the portion of the COMP gene encoding the fourth CaM-like repeat. Deletion of one of these aspartic acid codons, for residues 372–374 of the protein, was reported previously in one patient with typical PSACH (Briggs et al. 1995*a*). The same mutation was identified in another patient, also with typical PSACH, from family R95-109. Chondrocytes from the latter patient showed the characteristic lamellar RER inclusions of PSACH (data not shown).

Patient CM and affected individuals in families R95-107 and R93-237, all with typical PSACH, were heterozygous for point mutations predicted to result in a single–amino acid substitution for glycine 440, within the sixth CaM-like repeat. In families R95-107 and R93-237, a G→A transition at the second position of the codon predicted a G440E substitution (table 2). The mutation could be identified on the basis of a slowly

Table 2

Mutations and Polymorphisms Identified in the COMP Gene in Patients with PSACH or MED

Family or Patient	Nucleotide Change	Exon	Residue Change	Domain	Phenotype
R95-109	ΔGAC 1139–1147	10	ΔD 372–374	CaM-like #2	PSACH
R95-108[a]	ΔGAC 1430–1444	13	ΔD 469–473	CaM-like #4	PSACH
CM	G→A at 1343	13	G440R	CaM-like #6	PSACH
R95-107[b]	G→A at 1344	13	G440E	CaM-like #6	PSACH
R94-344	A→G at 1383	13	N453S	CaM-like #7	MED Fairbanks
R83-130	C→T at 1779	16	T585M	COOH	Mild PSACH
R92-002	C→G at 1779	16	T585R	COOH	MED
R83-130[c]	G→A at 1780	16	Neutral polymorphism	COOH	. . .

[a] Also carried by families R89-238, R92-207, R95-110, and R91-64 and by patients BW and CM.
[b] Also carried by family R93-237.
[c] Also carried by family R92-002.

migrating heteroduplex in amplified genomic DNA (fig. 2). Despite the fact that the proband in family R93-237 was the offspring of a consanguinous union, the mutation was not detected in amplified DNA from the unaffected parents; this indicates that the PSACH phenotype was the result of a new mutation. In the family of CM, an individual patient, a G→A transition at the first position of the codon predicted a G440R substitution (data not shown). Sequence analysis of an amplified genomic DNA fragment from both parents of CM showed absence of the mutation; this demonstrates that the PSACH phenotype was produced by a new dominant mutation.

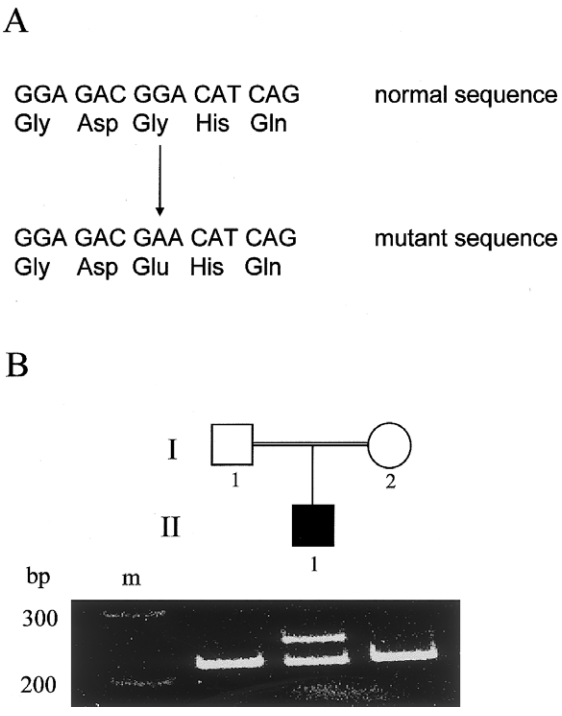

Figure 2 Sporadic COMP mutation (G440E) in family R93-237. *A,* Sequences of the normal and mutant alleles are summarized. *B,* PAGE of a PCR-amplified genomic DNA fragment containing the mutation in the proband and in the parents. Lane 1 (m) shows size markers, with sizes (in bp) indicated on the left.

In families R83-130 and R92-002, who have dominantly inherited forms of mild PSACH and MED, respectively, we previously reported linkage of the disease phenotypes to markers specific for the COMP locus on chromosome 19, and we excluded linkage to markers for COL9A1 and COL9A2 (Briggs et al. 1995*b*). In both families, we identified point mutations that are predicted to result in the substitution of threonine 585 in the carboxyl-terminal (COOH) domain of the protein. According to the previously published sequence (Newton et al. 1994) and our own data, the codon for threonine 585 is ACG. In family R83-130 (individual IV-2), we identified four clones with the sequence ATG and one clone with the sequence ACA; the C→T transition predicted a T585M substitution, whereas the G→A transition was a silent polymorphism (fig. 3). In family R92-002 (individual II-2), we identified three clones with the sequence AGG and three clones with the sequence ACG; the C→G transversion predicted a T585R substitution (fig. 3). For all members of the two families, analysis of PCR-amplified DNA from exon 16, by means of direct sequence determination, confirmed the sequences of the polymorphic alleles and demonstrated that the mutation in each family cosegregated with the phenotype, without exception (fig. 3).

In MED-Fairbank family R94-344, the disorder cosegregated with markers from the COMP region of chromosome 19, and linkage to COL9A1 and COL9A2 was excluded (Briggs et al. 1995*b*). We identified an A→G transition within the COMP gene that implied the substitution of a conserved asparagine residue by serine (N453S), in the seventh CaM-like repeat (fig. 4). The mutation created a *Bfa*I restriction endonuclease cleavage site, and analysis of amplified DNA from family members showed that the mutation cosegregated with the phenotype (fig. 4).

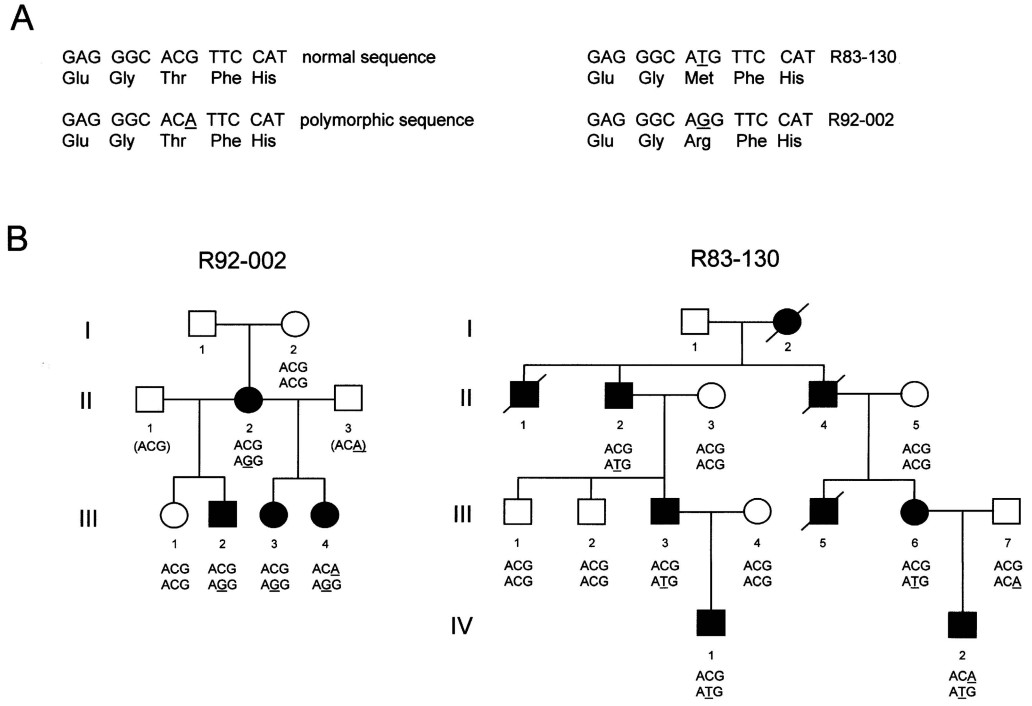

Figure 3 COMP mutations in MED family R92-002 and in mild-PSACH family R83-130. *A,* Summary of sequences. The normal (reported) and polymorphic sequences are shown on the left, and the sequences of the abnormal alleles in each family are shown on the right. *B,* Cosegregation of the mutation with the phenotype. Results of direct sequence analysis of a PCR-amplified DNA fragment, at codon 585, altered by the mutation and/or polymorphism. The mutation or polymorphic sequence is underlined.

Discussion

Among the 22 families with typical PSACH—20 reported here and 2 described in a previous report (Briggs et al. 1995*a*)—7 (31.8%) had the same mutation, an in-frame 3-bp deletion eliminating one of five consecutive aspartate codons for residues 469–473 of the protein. Deletion of one of three consecutive aspartate codons (for residues 372–374) from the fourth CaM-like repeat has now been identified in two patients, one described in a previous study (Briggs et al. 1995*a*) and the other described here. Thus, overall deletion mutations within the CaM-like–repeat domain were seen in 9 (40.9%) of the 22 patients with typical PSACH.

The data presented here also demonstrate that point mutations in the CaM-like domain can produce typical PSACH. In three unrelated individuals, we identified two different point mutations in the codon for glycine 440 that result in its substitution by either arginine or glutamic acid. The severity of the phenotype produced by these mutations suggests that this residue plays a major role in determining the structure and/or function of the CaM-like domain of COMP. Although the three-dimensional structure of the CaM-like domain of COMP is unknown, the sequence of paramecium CaM shows that glycine residues occur primarily at two positions (Kink et al. 1990): (1) they are adjacent to aspartic acid or asparagine residues within the calcium-binding pockets, or (2) they are positioned to allow CaM to form hydrogen-bonded turns that separate neighboring helical regions, and, as a result, they contribute to the secondary structure of the protein. We speculate that glycine 440 is positioned to form a hydrogen-bonded turn that, if disrupted, has a major effect on the overall structure of this region of the protein, in that it affects the relative positioning of calcium-binding pockets.

The N453S substitution identified in MED Fairbank family R94-344 correlates well with the mutations previously reported to produce MED phenotypes. These mutations result primarily in substitution of conserved aspartic acid and asparagine residues within the CaM-like domain, residues that are expected to line the calcium-binding pockets and to bind calcium by charge-charge interactions. The observation that such mutations usually produce MED provides support for our hypothesis (Briggs et al. 1995*a;* Cohn et al. 1996) that these mutations decrease the ability of individual calcium-binding pockets to bind calcium, without producing major effects on the structure of this region of the molecule.

In two families, one with mild PSACH (R83-130) and a second with a form of MED (R92-002), different substitutions for residue 585, in the carboxyl-terminal glob-

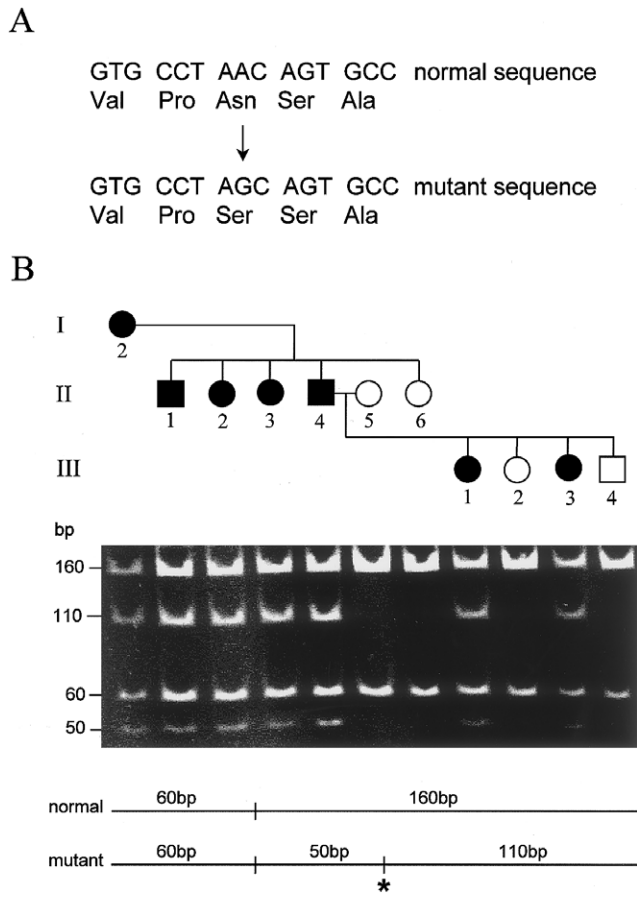

Figure 4 COMP mutation in family R94-344. *A*, Summary of sequence analysis predicting an N453S substitution. *B*, Cosegregation of the mutation with the phenotype. PAGE of a *Bfa*I-cleaved amplified–genomic DNA fragment containing the mutation. The pedigree is shown above, with the numeric designations of generations and individuals as described in the legend to figure 1. A line diagram of the amplified fragment, with the locations of cleavage sites for *Bfa*I and the sizes of the resulting fragments, is shown below. The location of the *Bfa*I site created by the mutation is indicated by an asterisk (*).

ular domain of COMP, were identified. The mild-PSACH family has typical radiographic features of the spine, with anterior beaking of the vertebral bodies during childhood and both metaphyseal and epiphyseal abnormalities. However, affected individuals in this family exhibit either normal height or only mild short stature. In contrast, the phenotype in the family with MED—with primarily epiphyseal irregularities and a normal spine—does not exhibit the radiographic features of PSACH, but the only affected adult in the family is of short stature. The clinical presentation of these families and the identification of COMP-gene mutations demonstrate phenotypic overlap between MED and mild forms of PSACH.

The mutations in the carboxyl-terminal domain indicate that this region has an essential role in the structure and/or function of COMP. Although the precise role is unknown, comparisons with the other members of the thrombospondin family of proteins provide some suggestions. Recent studies have shown that the carboxyl-terminal domain of thrombospondin 1 contains a non–RGD-dependent cell-binding domain based on the sequence RFYVVMWK (Gao and Frazier 1994; Tsao and Mousa 1995). This interaction, between thrombospondin 1 and many cell types, appears to be mediated by CD47 (Gao et al. 1996), an integrin-associated protein (IAP) that can bind to integrin $\alpha v\beta 3$. Tuckwell et al. (1994) determined the integrin profile of a human chondrosarcoma cell line, HCS-2/8 (Takigawa et al. 1989), identifying $\alpha v\beta 3$ as one of the expressed integrins. The sequence SFYVVMWK is present in the carboxyl-terminal domain of COMP (residues 608–615), suggesting a similar role for this sequence in IAP $\alpha v\beta 3$–mediated chondrocyte interactions. Interestingly, in vitro binding studies have shown that primary bovine chondrocytes can attach to purified bovine COMP (DiCesare et al. 1994), but the mechanism of this interaction is unknown. Threonine 585 is 23 amino residues terminal to this putative cell attachment site and may be involved in determining the secondary structure of the domain. Future studies will examine the possibility that the threonine 585 mutations affect the ability of COMP to interact with chondrocytes.

Mutations were not identified in 8 PSACH patients or in 20 MED patients. For PSACH, linkage studies have shown absence of locus heterogeneity (Hecht et al. 1993), so the mutations in the negative PSACH patients are likely to result either from the known inefficiencies of mutation detection by SSCP or from the possibility that the mutations are in COMP exons not examined in this study. For MED, the mutations may also be located in genes other than COMP, including the type IX collagen genes (Briggs et al. 1994; Muragaki et al. 1996).

The data presented here show that a variety of COMP structural mutations can produce a continuum of disease, ranging from a typical PSACH to a mild MED-Ribbing phenotype. Mutations predicted to disrupt the secondary structure of the CaM-like domain of COMP and to impede its secretion produce moderate to severe forms of PSACH. Mutations predicted to have milder effects on the secondary structure of the protein produce phenotypes at the MED end of the clinical spectrum.

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