Mutations in Cartilage Oligomeric Matrix Protein Causing Pseudoachondroplasia and Multiple Epiphyseal Dysplasia Affect Binding of Calcium and Collagen I, II, and IX*

Jochen Thur‡§, Krisztina Rosenberg‡‖, D. Patric Nitsche‡‡, Tero Pihlajamaa**,
Leena Ala-Kokko***, Dick Heinegård‡‡‖, Mats Paulsson‡‡‡, and Patrik Maurer‡‡§ §§

From the ‡Institute for Biochemistry, Medical Faculty, University of Cologne, D-50931 Köln, Germany,
*Department of Cell and Molecular Biology, Section for Connective Tissue Biology, Lund University,
S-22100 Lund, Sweden, and **Collagen Research Unit, Biocenter and Department of Medical Biochemistry,
University of Oulu, FIN-90014 Oulu, Finland

Mutations in type 3 repeats of cartilage oligomeric matrix protein (COMP) cause two skeletal dysplasias, pseudoachondroplasia (PSACH) and multiple epiphyseal dysplasia (MED). We expressed recombinant wild-type COMP that showed structural and functional properties identical to COMP isolated from cartilage. A fragment encompassing the eight type 3 repeats binds 14 calcium ions with moderate affinity and high cooperativity and presumably forms one large disulfide-bonded folding unit. A recombinant PSACH mutant COMP in which Asp-469 was deleted (D469A) and a MED mutant COMP in which Asp-361 was substituted by Tyr (D361Y) were both secreted into the cell culture medium of human cells. Circular dichroism spectroscopy revealed only small changes in the secondary structures of D469A and D361Y, demonstrating that the mutations do not dramatically affect the folding and stability of COMP. However, the local conformations of the type 3 repeats were disturbed, and the number of bound calcium ions was reduced to 10 and 8, respectively. In addition to collagen I and II, collagen IX also binds to COMP with high affinity. The PSACH and MED mutations reduce the binding to collagens I, II, and IX and result in an altered zinc dependence. These interactions may contribute to the development of the phenotypes and may explain why MED can also be caused by mutations in collagen IX genes.

Pseudoachondroplasia (PSACH)1 and multiple epiphyseal dysplasia (MED) constitute two autosomal dominantly inherited forms of osteochondrodysplasias characterized by severe to moderate short limb dwarfism with normal skull. Their clinical features include pronounced joint laxity and limitation of joint movement. The major clinical complication is caused by premature osteoarthritis of the weight-bearing joints (1). Both syndromes exhibit considerable clinical heterogeneity and show a broad phenotypical overlap with PSACH at the severe end of the disease spectrum and MED at the mild end of the disease spectrum (2, 3). Genetic linkage to chromosome 19 was recently demonstrated for both mild and severe forms and was soon followed by the identification of mutations in cartilage oligomeric matrix protein (COMP) that cause both PSACH and MED (4, 5).

COMP is a secreted pentameric glycoprotein built from modular units and belongs to the thrombospondin protein family (6–8). A coiled-coil domain at the amino terminus mediates the pentamerization resulting in a bouquet-shaped subunit arrangement (7, 9). It is followed by four epidermal growth factor (EGF)-like domains, a region encompassing eight so-called thrombospondin type 3 (T3) repeats and a carboxyl-terminal globular domain. COMP is found in cartilage, ligaments, and tendons (6, 10). Cells from synovial tissue express COMP in culture (11–13). Although COMP release to synovial fluid and blood plasma can serve as a diagnostic marker in early rheumatoid arthritis and osteoarthritis (14, 15) and increases after traumatic injury (16–19), its precise function is not established. The binding of COMP to collagens I and II with high affinity provides indications for structural roles (20).

All mutations in the COMP gene, which are known to cause PSACH or MED, result in either a single amino acid substitution, a small deletion, or an insertion of one or two amino acids (4, 5, 21–32). Thus far, no premature stop codon has been found. Most (44 of 48) known mutations in COMP affect residues in the T3 repeats, whereas 4 mutations are located in the carboxyl-terminal domain. 72% of the mutations affect acidic residues. For thrombospondin-1, it was postulated that the region encompassing the T3 repeats is responsible for the binding of 13 calcium ions (33). By analogy, it was speculated that the mutations of acidic residues in the T3 repeats may disturb the calcium binding, folding, and stability of COMP. Large inclusions in the endoplasmic reticulum of PSACH and MED patient chondrocytes contain COMP, collagen IX, and aggrecan and point to a destabilizing effect of the mutations. However, the inclusions were found only in chondrocytes and not in tenocytes and ligament cells (24, 34–36).

MED also shows a genetical heterogeneity. This clinically defined entity is caused not only by mutations in COMP but also by mutations in the COL9A2 and the COL9A3 genes that
encode the α2 and α3 chains of collagen IX, respectively (37–41). The mutations cause skipping of exon 3 for both chains and lead to collagenous domains with in-frame deletions of 12 amino acids. Thus far, no mutations have been reported for the α1 chain of collagen IX that complements the α2 and α3 chains in the heterotrimeric nonfibrillar collagen IX (42, 43). Collagen IX is a structural component of cartilage and was suggested to play a stabilizing role in collagen fibrils (44). The findings that mice expressing α1(IX) collagen chains with a central deletion develop a mild dwarfism and osteoarthriti (45) and that mice lacking collagen IX develop late-onset osteoarthriti (46, 47) supported this role.

To understand the consequences of mutations in COMP that cause PSACH and MED, we used recombinant COMP and several fragments thereof to demonstrate that the T3 repeats are responsible for calcium binding and that calcium binding is affected by the mutations. We also show that mutated COMP is still able to bind to collagens, but with altered zinc dependence.

The fact that COMP also binds to collagen IX and that this binding is interfered with by the mutations points to a common mechanism for the development of phenotypes in patients affected in either the COMP or collagen IX gene.

MATERIALS AND METHODS

Recombinant Expression of COMP in 293 Cells—The cDNA clone coding for full-length COMP (8) was a kind gift of A. Oldberg (Lund University, Lund, Sweden) and served as the template for PCR amplification of the different cDNA fragments. The cDNA coding for COMP lacking the signal peptide (rCOMP) was amplified according to standard protocols with Expand Polymerase (Roche Diagnostics) with primer 1 (5′-GCCCGCTAGCCGAGGCGGACTGCCC-3′) and primer M1 (5′-CAATGACCTGCGCGCTAGCCGCTACGAG-3′) (see Table 1). The EGF-T3 cDNA encoding residues 272–524 was generated with primer 3 (5′-GGTCTCTAATACTTCTGGTCATCATTC-3′) and primer M2 (5′-CAATGACCTGCGCGCTAGCCGCTACGAG-3′) and the T3-D361Y cDNA encoding residues 268–524 was amplified with primer 4 (5′-GCCCGCTAGCCGAGGCGGACTGCCC-3′) and primer M2. The PCR products were cloned in pUC18 linearized with EcoRV. Excision of the Nhel/NotI fragments and ligation into the pCEP-Pu expression vector (48) linearized with Nhel/NotI joined the 5′ end of the cDNA to the 3′ end, creating the construct for expression of truncated COMP. The cDNA was generated by PCR using primer 4 and mutagenesis primer 361m (5′-GGTCTCTAATACTTCTGGTCATCATTC-3′) with primer M2. A separate reaction was performed with primer M2 and primer 361c (5′-GCCCGCTAGCCGAGGCGGACTGCCC-3′). These PCR products served as the template for a final PCR with primer 4 and primer M2 to amplify T3-D361Y cDNA that was ligated into the pCEP-Pu. T3-D469A cDNA was generated using the combination of mutagenesis primer 361a (5′-GGTCTCTAATACTTCTGGTCATCATTC) and primer 4 and primer 469c (5′-GGTCTCTAATACTTCTGGTCATCATTC) with primer M2. Additional silent mutations created a novel Bst1107I in T3-D361Y and a new SphI site in T3-D469A to identify mutated clones. D361Y and D469A cDNA clones were constructed by digesting T3-D361Y and T3-D469A, respectively, with ClaI/BstBI and ligating these fragments into the ClaI/BstBI-digested rCOMP cDNA. Correct sequences of all clones were confirmed by sequencing (ABI Prism 377 DNA Sequencer; Applied Biosystems).

For stable expression, 5 × 10^5 human embryonic kidney cells (293-EBNA; Invitrogen) were electroporated with 3 μg of purified plasmid. The transfected cells were selected with 1 μg/ml puromycin and grown to confluence in Dulbecco’s modified Eagle’s medium Ham’s F-12 with 10% fetal calf serum (Life Technologies, Inc.). Secretion of recombinant proteins into the medium was confirmed by SDS-PAGE of the cell-free supernatant. The proteins were resolved by SDS-PAGE with or without prior reduction of disulfide bonds. Amino-terminal sequencing on an ABI473A/476A Sequencer (PerkinElmer Life Sciences) confirmed the identity of the proteins.

Solid-phase Ligand Binding Assays—Collagen I and II were purchased from Sigma. Collagen I had been isolated after papain digestion of the bovine nasal septum and collagen II had been isolated from bovine nasal septum. Each collagen was dissolved in 0.5 M acetic acid. Recombinant collagen IX was produced as described previously (49). Ligand binding studies were performed in solid-phase assays with collagens (2 μg/ml in 0.1 M acetic acid) coated to 96-well plates (Maxisorb; Nunc) overnight at 20 °C. After washing with 0.05% Tween 20 in 50 mM Tris, pH 7.4, 150 mM NaCl (TBS), the wells were blocked for 1.5 h with 10 mg/ml bovine serum albumin in TBS at room temperature. After washing the wells were incubated for 1 h at room temperature with ligands tCOMP, rCOMP, D361Y, and D469A diluted in TBS containing 0.5 mM ZnCl₂. The ligands were used at different concentrations for the determination of affinity. Nonspecific binding was controlled for each ligand concentration in wells lacking a collagen coat but otherwise treated identically. In separate experiments, the zinc-dependent binding was measured by incubating ligands (5 μM diluted in TBS containing 0.5 mM ZnCl₂) with different concentrations. Also the washing buffers were supplemented with ZnCl₂. Bound tCOMP, rCOMP, D361Y, and D469D were detected using a polyclonal antiserum against COMP (50) followed by peroxidase-coupled swine anti-rabbit IgG (DAKO) using tetramethylbenzidine as chromogenic substrate, and absorbance was recorded at 450 nm. Identical titers of the antibodies against wild-type and mutant COMP were confirmed in enzyme-linked immunosorbent assay titrations in which wild-type and mutant COMP were coated onto plastic and detected with different dilutions of the polyclonal antiserum.

Surface Plasmon Resonance Assay —The BIAcore 2000 system (BIAcore, Uppsala, Sweden) was used to characterize the interaction between wild-type and mutant COMP and collagens. Immobilization of tCOMP, rCOMP, D361Y, and D469A to the carboxymethylated matrix of the CM5 sensor chip was performed as described previously (20). The immobilization resulted in 9,100 resonance units for tCOMP, 4,600 resonance units for rCOMP, 3,300–3,600 resonance units for D361Y, and 3,400–5,200 resonance units for D469A. A blank surface was used as control. Binding was determined at different concentrations of collagen I (50 nM) and collagen II (5–88 nm). The collagens were added to 20 mM Hepes, pH 7.4, 150 mM NaCl, 0.005% Tween 20 containing 0.05 mM zinc chloride before injection to avoid fiber formation. A flow rate of 80 μl/min was used. BIAevaluation software version 3.0 was used to calculate K_d of the binding. Only curves in which the result applied in a signal response of more than 10 resonance units were used for calculations.

CD Spectroscopy—Far-UV CD spectra of the far UV region were recorded in a thermostated quartz cell of 1 mm optical path length in a Jasco 715 CD spectrophotometer at 25 °C. Spectra were measured with the proteins dissolved in TBS (less than 5 μM Ca²⁺) and after the addition of 0.25 and 1.5 mM CaCl₂. Reversal of the conformational change was examined after the addition of 4 mM EDTA to the Ca²⁺-containing samples. Mean molar ellipticities [θ] expressed in degrees cm² dm³ mol⁻¹ were calculated on the basis of a mean residue molecular mass of 110 Da. Five spectra were accumulated to improve the signal/noise ratio, and the spectra of buffers were subtracted. The percentage change in CD at 215 nm was calculated as Δ[θ]215 = 100 × ([θ]_Ca²⁺ - [θ]_EDTA)[[θ]_Ca²⁺ - [θ]_EDTA], representing the signal at Ca²⁺ saturation, and [θ]_EDTA representing the CD signal in the presence of excess EDTA. Calcium titrations were performed by the addition of small aliquots of CaCl₂ stock solution followed by incubation for 5 min and recording of the CD signal at 215 nm or recording of spectra. Secondary structure analysis was performed using the variable selection method SelCon (51).

Fluorometry and Ca²⁺ Titrations—Fluorometry was measured with a PerkinElmer Life Sciences LS50B spectrophotometer in 10-mm pathlength cuvettes containing 20 μM CaCl₂ at 25 °C. The samples were excited at 280 nm for all recombinant proteins (1 μM). Emission maxima were between 346 and 353 nm. Spectra were recorded in TBS (less than 5 μM Ca²⁺) in the presence of 2 mM CaCl₂ and after the subsequent addition of 4 mM EDTA. The percentage change in fluorescence intensity at 350 nm was calculated as ΔF350 = 100 × (F_Ca²⁺ - F_EDTA)[F_Ca²⁺ - F_EDTA], representing the signal at Ca²⁺ saturation, and F_EDTA representing the signal in the presence of excess EDTA. Calcium titrations were performed by the addition of small aliquots of CaCl₂ stock solution followed by incubation for 5 min and recording of the CD signal at 215 nm or recording of spectra. Secondary structure analysis was performed using the variable selection method SelCon (51).
fluorescence signal in the presence of excess EDTA. Calcium titrations were performed as described above.

**Equilibrium Dialysis**—The number of bound calcium ions per COMP molecule was determined by equilibrium dialysis in microdialysis chambers (Membrapure, Bodenheim, Germany). 150 μl of protein (10 μM) dissolved in TBS was dialyzed against 150 μl of TBS containing 0.2 μCi of ⁴⁰CaCl₂ (Amersham Pharmacia Biotech) and different ⁴⁰CaCl₂ concentrations. After 16–20 h, 10-μl aliquots were removed from each dialysis chamber and counted in a scintillation counter. The number of bound calcium ions B was calculated as \( B = cpm/c_{\text{total}} \times n_{\text{protein}} \) with \( cpm \) representing the difference in radioactivity between both dialysis chambers, \( c_{\text{total}} \) representing the sum of the activities, \( n_{\text{protein}} \) representing the number of calcium molecules in the system. The free calcium concentration was calculated as the difference between the total and the bound calcium concentration. Protein concentrations were determined according the method of Gill and von Hippel (64).

**Data Evaluation**—The degree of saturation \( Y \) was calculated from the signals \( S \), corrected for dilutions, as \( Y = (S - S_0)/(S_C - S_0) \), where \( S_C \) and \( S_0 \) represent the signals at Ca²⁺ saturation and 0 calcium²⁺, respectively. The free Ca²⁺ concentrations were calculated from \( Y \) and the corresponding total calcium concentration \( Ca^{2+}_\text{tot} \) to: \( Ca^{2+}_\text{free} = Ca^{2+}_\text{tot} \times Y \times n \times F/\text{tot} \) with \( n \) representing the number of calcium binding sites/molecule, \( F/\text{tot} \) representing the total protein concentration, and \( n \times F/\text{tot} \) representing the total concentration of binding sites present. We initially used \( n = 13 \) for the number of Ca²⁺ binding sites/molecule, as determined for thrombospondin-1 (33). Because the protein concentrations used were much lower than \( Ca^{2+}_\text{tot} \), \( n \) had only a negligible effect on \( Ca^{2+}_\text{free} \). Thus, variation of \( n \) between 10 and 20 and between 3 and 10 for the mutant T3 repeats had no significant effect on the calculated \( K_d \) values and Hill coefficients. The degree of saturation \( Y \) in a model including two independent classes of binding sites can be described by \( Y = \alpha \times Y_1 + (1 - \alpha) \times Y_2 \) with \( Y_1 \) and \( Y_2 \) representing the degrees of saturation of the first and second class of binding sites, and the parameter \( \alpha \) representing the percentage contribution of the signal induced by saturation of the first class of binding sites to the whole signal. Using the Hill equation to describe cooperativity between the sites within each class of binding sites \( Y \) is given by

\[
Y = a \times \left( \frac{[Ca]^{n\alpha}}{K_{H1}^{n\alpha} + [Ca]^{n\alpha}} \right) \times \left( 1 - a \right) \times \left( \frac{[Ca]^{n\beta}}{K_{H2}^{n\beta} + [Ca]^{n\beta}} \right)
\]

(Eq. 1)

where \([Ca]\) represents the free Ca²⁺ concentration, \( K_{H1} \) and \( K_{H2} \) represent the equilibrium dissociation constants, and \( n_{\alpha} \) and \( n_{\beta} \) represent the Hill coefficients. The program Grafit (Erithacus software) was used to fit the model to the experimental data with nonlinear least-square fit procedures.

**Electron Microscopy**—For visualization by electron microscopy, wild-type and mutant COMP and collagen (0.1 μM) were dialyzed against 0.15 mM ammonium formate, pH 7.4. ZnCl₂ was added to give 0.5 mM zinc²⁺. The replicas were floated off onto distilled water, placed on freshly cleaved mica, dried in vacuum, and rotary shadowed with platinum/carbon (52). The replicas were floated off onto distilled water, picked up on 400-mesh copper grids, and examined in a Zeiss 902 electron microscope.

**RESULTS**

We expressed rCOMP as well as several fragments thereof in a eukaryotic expression system to ensure proper disulfide bond formation and posttranslational modifications. Because it was unclear whether the thrombospondin T3 repeats are independently folded, a construct EGF-T3, encompassing the fourth EGF domain plus the eight T3 repeats, and a construct T3, encompassing only the eight T3 repeats, were produced (Fig. 1). Furthermore, we introduced two mutations in the cDNA for COMP. The point mutation D361Y causes mutations D361Y and D469, respectively, and the single tryptophan (Trp-344; W) is indicated.

**Moderate Affinity and High Cooperativity**—A signal peptide targeted the recombinant proteins into cell culture media used to purify the proteins to homogeneity (Fig. 2). rCOMP was present in the culture medium as a pentamer of about 550 kDa, although bands migrating at lower molecular masses were also observed under nonreducing conditions. A band at 100 kDa was isolated, and amino-terminal sequencing revealed that the sequence started at amino acid 78 within the linker between the coiled-coil domain and the first EGF-like domain (results not shown). Apparently, a protease present either within the cells or in the culture medium is able to cleave the COMP subunits, yielding the cleaved carboxyl-terminal parts of 100 kDa and fragments with one to five intact subunits connected via the pentameric coiled-coil domain. The smaller fragments including the 100-kDa band could be removed chromatographically, and a preparation of pentameric COMP with a portion of COMP particles lacking the carboxyl-terminal part of one subunit was obtained. Under reducing conditions, this preparation showed a single band at 110 kDa (Fig. 2). Amino-terminal sequencing confirmed that that this band represents the full-length COMP. Electron microscopy after rotary shadowing confirmed the main pentameric structure of rCOMP. As seen earlier for tCOMP (7), five globular domains are connected by thin flexible strands to a central assembly domain (Fig. 3).

Folding of rCOMP was investigated by CD spectroscopy. CD spectra in the far UV region for rCOMP and tCOMP were indistinguishable and characteristic for a protein with a mixed β-sheet-random conformation and low α-helical content (Fig. 4). Contents of 14% α-helix, 33% β-sheet, and 53% β-turns and unordered conformation were calculated.

The addition of 0.25 mM calcium induced a small conformational change, whereas higher calcium concentrations induced an aggregation of rCOMP and tCOMP that leads to a loss of ellipticity due to light scattering (results not shown). The calcium-dependent conformational change was also observed for EGF-T3 (results not shown) and the T3 repeats alone (Fig. 4). For the smaller fragments, it could be reversed even in the presence of 1.5 mM calcium by the addition of excess EDTA.

Equilibrium dialysis revealed that 14 calcium ions bind to the T3 repeats at calcium saturation (Fig. 5). Results on the number of binding sites without cooperativity failed to adequately describe the binding curve. A good fit was achieved when cooperativity was allowed in the model, and a \( K_d = 0.15 \) mM and a Hill coefficient \( n_H = 4.9 \) were evaluated. The CD signal at 215 nm was used to monitor the calcium dependence of the conformational change of the T3 repeats. A biphasic titration curve was observed in which 80% of the signal were reached by the addition of 1 mM calcium, and further addition to 20 mM resulted in a second
signal change (Fig. 6). Again, a good fit was achieved when cooperativity was allowed in the model and yielded an equilibrium dissociation constant $K_{d1} = 0.3$ mM and a Hill coefficient $n_{H1} = 3.7$ that describes the conformational change by calcium binding at low calcium concentrations. A second $K_{d2} = 4.1$ mM with low cooperativity (Hill coefficient $n_{H2} = 1.2$) was obtained for the signal change at high calcium concentrations (Table I). No significant difference in calcium affinity was observed for the EGF-T3 fragment (results not shown).

COMP Carrying Mutations that Cause PSACH or MED Is Folded and Secreted by 293 Cells—In serum-free cell culture media, we could readily detect both mutated full-length COMP D361Y and D469Δ, which were subsequently purified to homogeneity (Fig. 2). We observed neither signs of intracellular inclusions or cell death nor significant differences in protein yields as compared with the wild-type proteins. Electron microscopy revealed a pentameric structure with five globular domains connected to a central assembly domain similar to that seen for rCOMP and tCOMP (Fig. 3). We used CD spectroscopy to analyze whether secondary structure is maintained in mutated COMP molecules. Compared with tCOMP and rCOMP, only a very small difference in ellipticity could be observed in the spectra (Fig. 4). Furthermore, the small conformational change upon the addition of calcium could also be observed for the D361Y and D469Δ mutant proteins, and higher calcium concentrations induced precipitation as seen with tCOMP and rCOMP. The small change in ellipticity and the precipitation precluded the determination of calcium affinity in the full-length proteins.

Calcium Binding Is Affected in Mutated COMP—To analyze the conformational change in greater detail, we introduced the D361Y and D469Δ mutations in the T3 repeats. Again, both proteins were expressed and secreted by the 293 cells (Fig. 2). The number of calcium binding sites in T3-D361Y and T3-D469Δ was reduced to 8 and 10, respectively, but affinity and cooperativity of the remaining sites were only slightly affected (Fig. 5; Table I). Analysis of the CD spectra revealed pronounced conformational differences in the presence and absence of calcium compared with wild-type T3 repeats (Fig. 4). Although both T3-D361Y and T3-D469Δ were able to reversibly bind calcium, titrations showed clear differences compared with wild-type T3 repeats (Fig. 4). The total change of ellipticity was slightly decreased in both mutants. 70% of signal of the D469Δ mutation corresponded to the cooperative sites with moderate calcium affinity ($K_{d1} = 0.28$ mM, Hill coefficient $n_{H1} = 3.7$), whereas only 55% of the signal was induced in T3-D361Y by the occupation of these calcium sites ($K_{d1} = 0.25$ mM, $n_{H1} = 4.5$). The loss of signal induced by the low calcium concentrations was accompanied by a larger signal change induced at millimolar calcium concentrations ($K_{d2} = 1.5$ and 2.7 mM, respectively; Table I). Thus, both mutations shift part of the calcium binding from moderate affinity to low affinity, which results in a decreased number of bound calcium ions at the physiological calcium concentration of 1 mM.

Calcium binding was also monitored by intrinsic fluorescence. Upon excitation at 280 nm, an emission maximum at 346 nm was observed for the calcium-saturated T3 repeats. This fluorescence is caused by Trp-344 within the third T3 repeat.
and half-maximal binding was obtained at 3.5 and 1.5 nM (Fig. 4). COMP showed concentration-dependent saturation curves, indicating an assay-style ligand-binding assay. Both tCOMP and rCOMP detected COMP binding in an enzyme-linked immunosorbent assay format (24, 43, 53, 54). We coated microtiter plates with collagen IX to test whether COMP also binds to collagen IX, which is found in tendons and ligaments to bone and which accumulates together with COMP in the ER inclusions in MED and PSACH patients (20). This prompted us to investigate whether COMP also binds to collagen IX, which is found in cartilage as well as the fibrocartilaginous insertion sites of tendons and ligaments to bone and which accumulates together with COMP in the ER inclusions in MED and PSACH patients (20, 24, 43, 53, 54). We coated microtiter plates with collagen IX and detected COMP binding in an enzyme-linked immunosorbent assay-style ligand-binding assay. Both tCOMP and rCOMP showed concentration-dependent saturation curves, and half-maximal binding was obtained at 3.5 and 1.5 nm (Fig. 8, A and B). Control experiments with COMP added to bovine serum albumin-coated wells resulted in less than 5% of the absorbances obtained with collagen-coated wells even at high COMP concentrations. Binding was confirmed by surface plasmon resonance detection with COMP immobilized on the chip and collagen in solution (Fig. 9) as well as with immobilized collagens and COMP in solution (results not shown). To obtain $K_d$ values from the surface plasmon resonance curves, we fitted a Langmuir model for one-to-one interaction to the curves measured at different collagen concentrations. Multiple binding sites are present on both the collagens and the pentameric COMP, and the $K_d$ values are therefore an average of several binding sites. The average $K_d$ value for collagen IX binding was 32 nM (Table II). Furthermore, complex formation between COMP and collagen IX could be observed by electron microscopy (Fig. 10). The recombinant collagen IX forms a filamentous structure with a globular end representing the amino-terminal NC4 domain of the α chain. A sharp kink within the collagenous domain represents the NC3 domain, as shown for collagen IX isolated from tissue (55). COMP is bound to both ends of the collagen IX molecule and to two additional internal sites. One is located at the kink representing the NC3 domain and the second internal site is found at a distance of about 160 nm and is thus in the vicinity of the NC2 domain. In some complexes, resolution was sufficient to reveal that COMP interacts by its globular domains with the collagen IX (Fig. 10). Identical saturation curves for tCOMP and rCOMP were also obtained with collagen I (Fig. 8, A and B), corroborating the functionality of the recombinant proteins.

**COMP binds to Collagen IX**—COMP binding to collagen I and II was reported recently (20). This prompted us to investigate whether COMP also binds to collagen IX, which is found in cartilage as well as the fibrocartilaginous insertion sites of tendons and ligaments to bone and which accumulates together with COMP in the ER inclusions in MED and PSACH patients (24, 43, 53, 54). We coated microtiter plates with collagen IX and detected COMP binding in an enzyme-linked immunosorbent assay-style ligand-binding assay. Both tCOMP and rCOMP showed concentration-dependent saturation curves, and half-maximal binding was obtained at 3.5 and 1.5 nm (Fig. 8, A and B). Control experiments with COMP added to bovine serum albumin-coated wells resulted in less than 5% of the absorbances obtained with collagen-coated wells even at high calcium concentrations. Binding was confirmed by surface plasmon resonance detection with COMP immobilized on the chip and collagen in solution (Fig. 9) as well as with immobilized collagens and COMP in solution (results not shown). To obtain $K_d$ values from the surface plasmon resonance curves, we fitted a Langmuir model for one-to-one interaction to the curves measured at different collagen concentrations. Multiple binding sites are present on both the collagens and the pentameric COMP, and the $K_d$ values are therefore an average of several binding sites. The average $K_d$ value for collagen IX binding was 32 nM (Table II). Furthermore, complex formation between COMP and collagen IX could be observed by electron microscopy (Fig. 10). The recombinant collagen IX forms a filamentous structure with a globular end representing the amino-terminal NC4 domain of the α chain. A sharp kink within the collagenous domain represents the NC3 domain, as shown for collagen IX isolated from tissue (55). COMP is bound to both ends of the collagen IX molecule and to two additional internal sites. One is located at the kink representing the NC3 domain and the second internal site is found at a distance of about 160 nm and is thus in the vicinity of the NC2 domain. In some complexes, resolution was sufficient to reveal that COMP interacts by its globular domains with the collagen IX (Fig. 10). Identical saturation curves for tCOMP and rCOMP were also obtained with collagen I (Fig. 8, A and B), corroborating the functionality of the recombinant proteins.

**Binding of COMP to Collagens I, II, and IX Is Affected by PSACH and MED Mutations**—Collagen binding of COMP was previously shown to depend on the presence of zinc ions (20).
We therefore compared the binding to collagens at different zinc concentrations. Whereas half-saturation binding of rCOMP to collagen I was observed at 10 μM zinc, significantly more zinc was needed to induce binding of D361Y and D469Δ (K_{d3} = 20 and 40 μM, respectively; Fig. 11A). Similarly, higher zinc concentrations were needed to induce binding of D361Y and D469Δ to collagen II and IX compared with wild-type COMP (Fig. 11, B and C). However, when collagen binding was measured at higher zinc concentrations, similar binding profiles and affinities of wild-type and mutated COMP for collagen were obtained (Figs. 8 and 9; Table II). We also quantitated COMP binding using electron microscopy. Analysis of more than 300 COMP molecules showed that in 1 mM EDTA, only 28% were bound to collagen IX, whereas 51% were bound in the presence of 1 mM zinc. The only partial binding in zinc results from low concentrations of both binding partners used to avoid nonspecific juxtaposition of molecules on the grid and is in agreement with K_{d} values obtained by the surface plasmon resonance studies.

The zinc dependence was further analyzed using proteolysis of COMP. Chymotrypsin completely degraded COMP when no cations were present. Calcium and zinc were able to partially protect cleavage sites. However, different fragments were obtained in the presence of zinc compared with calcium (Fig. 12).

To further investigate the cleavage site obtained in the presence of zinc, the corresponding band was excised from the gel, digested with trypsin, and analyzed by sequencing of some of the resulting peptides using ESI-Q-TOF (electrospray ionization quadrupole time-of-flight) mass spectrometry. The peptide encompassing the amino terminus of COMP and the peptides from the T3 repeats were identified, whereas carboxyl-terminal peptides were lacking. This demonstrates a carboxyl-terminal cleavage presumably in the carboxyl-terminal domain. An effect of zinc on the conformation of the carboxyl-terminal domain is also in agreement with CD titrations, showing that, in contrast to calcium, 1 mM zinc did not affect the conformation of the T3 repeats (results not shown).

### DISCUSSION

COMP is a modular protein composed of different types of domains. Thus far, the three-dimensional structure has been characterized only for the coiled-coil domain of COMP (9). The structures of the EGF-like domains can be predicted from homologous domains in other proteins. On the other hand, reasonable structural models are available for neither the T3 repeats nor the carboxyl-terminal domain. Also, the knowledge of COMP function is limited to the recent demonstration of interactions with collagens I and II (20). Due to this lack of structural and functional data, the consequences of PSACH- and MED-causing mutations and hence the molecular pathogenesis of the disease are unknown. To learn more about structural and functional alterations, we expressed recombinant T3 repeats either alone, in connection with the fourth EGF-like domain, or within the full-length protein. CD spectra, electron

![Fig. 6. Calcium affinity and cooperativity of type 3 repeats and their mutants.](chart)

Increasing concentrations of calcium were added to the proteins dissolved in TBS, and circular dichroism was recorded at 215 nm. Ellipticity was converted into saturation Y to the proteins dissolved in TBS, and circular dichroism was recorded at 215 nm. Increasing concentrations of calcium were added and their mutants.

**Table I**

| Equilibrium dialysis | Circular dichroism | Fluorescence |
|----------------------|--------------------|--------------|
| B_{max}^{a} | K_{d}^{a} | n_{H1}^{a} | Δ[θ]^{b} | K_{d1}^{b} | n_{H1}^{b} | Signal 1^{d} | K_{d2}^{d} | n_{H2}^{d} | ΔF^{e} | K_{d1}^{e} | n_{H1}^{e} | Signal 1^{f} | K_{d2}^{f} | n_{H2}^{f} |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| T3 | 14.1 | 0.15 | 4.9^{e} | 50 | 0.30 | 3.7 | 80 | 4.1 | 1.2 | 430 | 0.24 | 5.9 | 94 | 1.25 | 2.0 |
| T3-D361Y | 8.1 | 0.18 | 1.7^{e} | 40 | 0.25 | 4.5 | 55 | 2.7 | 1.5 | 20 | 1.12 | 3.8 | 98 | 0.69 | 1.9 |
| T3-D469A | 10.4 | 0.14 | 2.1^{e} | 40 | 0.28 | 3.7 | 70 | 1.5 | 2.8 | 280 | 0.24 | 8.0 | 80 | 0.69 | 1.9 |

*a* Number of bound calcium ions at saturation.

*b* Percentage change of the CD signal at 215 nm.

*c* Equilibrium dissociation constants K_{d} and Hill coefficients n_{H1} were derived from a model with two classes of cooperative binding sites using circular dichroism at 215 nm as signal.

*d* Percentage contribution of the signal corresponding to calcium binding sites with K_{d1} and n_{H1}.

*e* Percentage change of the fluorescence signal at the emission wavelength of 350 nm upon excitation at 280 nm.

*f* Equilibrium dissociation constants and Hill coefficients were derived from a model with two classes of cooperative binding sites using intrinsic fluorescence as signal.

*g* Differences in Hill coefficients n_{H1} are not significant due to low signal to noise ratios.
Effects of PSACH and MED Mutations in COMP

**FIG. 7.** Fluorescence spectra and calcium titrations of wild-type and mutant type 3 repeats. Fluorescence emission spectra were recorded at an excitation of 280 nm in TBS in the absence of calcium (solid lines) and in the presence of 1.5 mM calcium (dotted lines) for T3 (A), T3-D361Y (B), and T3-D469A (C). Calcium titrations were monitored upon excitation at 280 nm and emission at 350 nm (D). Fluorescence signals were converted into saturation recorded upon excitation at 280 nm and emission at 350 nm (D). Fluorescence equilibrium dissociation constants, T3-D361Y represented by $\text{D}_{\text{T3-D361Y}}$, and T3-D469A represented by $\text{D}_{\text{T3-D469A}}$. Best fit curves obtained from a binding model including cooperativity are shown as solid lines.

**TABLE II**

Collagen binding of wild-type and mutant COMP

| Collagen | $K_d$ | $K_d$ | $K_d$ | $K_d$ |
|----------|-------|-------|-------|-------|
| I | (nM) | (nM) | (nM) | (nM) |
| tCOMP | 0.6 ± 0.1 | 3.5 ± 0.1 | 13 ± 5 | n.d. |
| rCOMP | 0.6 ± 0.1 | 1.5 ± 0.1 | n.d. | 32 ± 7 |
| D361Y | 0.4 ± 0.1 | 0.9 ± 0.1 | 10 ± 3 | 16 ± 5 |
| D469A | 0.4 ± 0.1 | 0.9 ± 0.1 | 11 ± 3 | 16 ± 3 |

Concentrations needed for half-maximal saturation $K_d$, and average equilibrium dissociation constants $K_d$ for the binding of COMP to pepsinized collagen I and full-length recombinant collagen IX were evaluated from binding measurements using enzyme-linked immunosorbent assay-style ligand-binding assays in the presence of 0.5 mM zinc or surface plasmon resonance in the presence of 0.05 mM zinc. n.d., not determined.

**Fig. 8.** Binding of COMP and mutant COMP to collagens I and IX in a solid-phase assay. Collagen I (solid lines) and IX (dotted lines) were coated overnight at 2 $\mu$g/ml in 0.18 M acetic acid onto plastic wells and incubated with 0–20 nM tCOMP (A), rCOMP (B), D361Y (C), and D469A (D) in TBS with 0.5 mM ZnCl$_2$ for 1 h. Bound COMP was detected using an antiserum to COMP followed by peroxidase-coupled antiserum against rabbit IgG using TMB as the chromogenic substrate and recording absorbance at 450 nm. Data are the means of quadruplicate determinations, and error bars show the mean absolute deviations.

**Fig. 9.** COMP binding to collagen IX detected by surface plasmon resonance assay. A, concentration dependence of collagen IX binding to immobilized rCOMP. B, COMP (solid lines), D361Y (dashed line), and D469A (dotted line) immobilized on a CM5 sensorchip were allowed to interact with 88 nM collagen IX in 20 mM Heps, pH 7.4, 150 mM NaCl in the presence of 0.05 mM ZnCl$_2$. A flow rate of 80 $\mu$l/min was used, and a volume of 40 $\mu$l of ligand was injected at 120 s.

**To analyze the effects of COMP mutations, we genetically engineered the COMP cDNA in such a manner that COMP with a D469A or a D361Y substitution was produced.** The mutations were chosen according to the severity of phenotypes observed for PSACH and MED patients. The D469A mutation causes PSACH with severe dwarfism, whereas the D361Y mutation causes MED with mild to severe osteoarthritis (4, 5, 21, 28–30). Both mutants were secreted by the human kidney cells used for expression, formed pentamers, and showed a folding similar to that of wild-type rCOMP and tCOMP. In addition, a similar conformational change upon calcium addition was observed at low calcium concentrations. Thus, in the context of full-length COMP, mutations in the T3 repeats have only subtle effects and do not produce an instability of the protein per se.

More detailed insight was obtained by the analysis of the recombinant T3 repeats. The findings that the T3 repeats can be expressed independently and that they bind calcium with high cooperativity indicate that they constitute an autonomous folding unit. Despite the uneven number of 17 cysteines, oligomer formation could not be detected. A mobility shift in SDS-PAGE between reducing and nonreducing conditions indicates the formation of intramolecular disulfide bonds within the T3 repeats (results not shown). CD and fluorescence spectroscopy revealed that calcium but not zinc can induce a reversible change of the conformation. Analysis of secondary structures of the T3 repeats indicated the presence of $\alpha$-helices and $\beta$-sheets. However, their elongated structure, multiple disulfide bonds, and acidic nature may invalidate these second-
ary structure predictions, which depend on the chosen standard protein data set (56). Attempts to crystallize the T3 repeats in the presence or absence of calcium have failed, perhaps indicating a highly flexible conformation. Calcium binding is strongly cooperative, and the affinity is high enough that the T3 repeats should be in the calcium-saturated form in the presence of physiological extracellular calcium concentrations. Interestingly, the mutations D469Δ and D361Y did not induce a breakdown of the cooperative system, and only 4 or 6 of the 14 calcium binding sites were lost. 14 calcium binding sites for wild-type COMP are in good agreement with the 13 sites reported for the homologous thrombospondin-1 (33). During the course of our study, Maddox et al. (65) reported that the mutation D446N in COMP, which causes PSACH, reduced the binding from 17 calcium ions to 8 calcium ions. In an additional study, Chen et al. (66) also introduced the deletion of D469 in COMP. Their mutant protein bound seven calcium ions, compared with nine calcium ions bound to the wild-type protein. The relative loss of about 25% of the calcium binding sites is similar to our results. Equilibrium dialysis results depend strongly on exact protein concentrations, and this is presumably the reason for the discrepancy in the absolute numbers. We experimentally determined extinction coefficients and used these to measure protein concentrations by UV spectroscopy, thus avoiding many of the pitfalls of other methods (64).

To gain more insight into the effect of mutations on the conformation, we used circular dichroism and fluorescence spectroscopy. Because Trp-344 fluorescence changes cooperatively upon calcium binding with $n_{1} \geq 6$, at least six calcium ions influence the local conformation of Trp-344, in agreement with the loss of six sites when the mutation is present in the neighboring repeat. However, affinity and cooperativity of the remaining intact sites are only slightly affected. The interference of the fluorescence change in repeat 3 by the D469Δ mutation, which is located in repeat 7, corroborates the model in which the eight T3 repeats do not represent individual autonomous repeats but form a larger disulfide-bonded folding unit. The point mutations causing PSACH and MED locally affect the affinity of several calcium ions but also influence the conformation of distant repeats. Thus far, no correlation between the type or location of the mutation and the severity of the disease has been found. The fact that the D361Y mutation, which causes mild MED, binds fewer calcium ions than the D469Δ mutation, which causes severe PSACH, demonstrates that the number of bound calcium ions does not correlate with the PSACH or MED phenotype.

We show that the mutations affect the binding of COMP to collagens I and II, the only COMP ligands described previously (20). Because the binding site for collagens is located in the carboxyl-terminal domain of COMP, the affected collagen binding of COMP with mutations in the T3 repeats suggests that the conformation of the carboxyl-terminal domain is influenced by the type 3 repeats and that this interaction is disturbed by PSACH and MED mutations. This is in agreement with the finding that mutations in the carboxyl-terminal domain can also cause PSACH and MED, respectively. Decreased arm length and different stokes radius seen in the mutant COMP also support this notion (65, 66). The interaction of COMP and collagens is dependent on the presence of zinc ions, and higher zinc concentrations are needed for efficient binding of mutant COMP. We also showed that zinc can directly affect the conformation of COMP, presumably by binding to the carboxyl-terminal domain. Zinc concentrations in synovial fluid are 9–25 μM (57), whereas the total zinc concentration in cartilage varies with age between 15 and 120 μM (58). In different layers and compartments of cartilage, such as the hypertrophic zone of the growth plate, a concentration of up to 1400 μM total zinc was detected (59). The mutations in COMP might thus shift the proportion of COMP molecules bound to collagens. We propose that the affected collagen binding of mutant COMP may contribute to the development of phenotypes of PSACH and MED patients. Although the precise mechanisms are still unknown, secreted mutant COMP might interfere with proper formation and organization of collagen I and II fibrils and thus affect structure and stability of cartilage, tendons, and ligaments. Such a dominant negative effect of secreted mutant COMP is also in agreement with the observation that mice deficient in COMP grow to normal length and do not develop severe osteoarthritis.3

The presence of inclusions in the ER points to an alternative mechanism for the pathogenesis of PSACH and MED. According to this hypothesis, COMP and additional proteins destined for secretion are retained in the ER, and the lack of these proteins in the extracellular matrix, together with an affected cellular function, could cause the phenotypes (5, 60). Thus far, the lack of antibodies specific for mutant COMP molecules has

---

2 E. Hohenester and P. Maurer, unpublished observations.

3 L. Svensson and Å. Oldberg, personal communication.
hampered the study of the fate of such COMP molecules. Patient chondrocytes, tenocytes, and ligament cells are able to secrete pentameric COMP in vitro (61, 62). For chondrocytes, this is presumably related to their dedifferentiation in monolayer cell culture (60). In vivo, COMP has been shown to be present extracellularly in tendons of a PSACH patient, in agreement with the lack of inclusions in tenocytes (24). Thus, at least in tendons, mutant COMP is secreted and might cause a dominant negative effect. In contrast, COMP was not found (24) or was found in reduced amounts (62) in the extracellular matrix of patient cartilage in immunohistochemical studies. This was interpreted as being due to the retention in the endoplasmic reticulum of chondrocytes but could also be caused by an only weak anchorage of secreted mutant COMP in the extracellular matrix or subsequent loss of COMP during the staining procedure. Additional mechanisms, such as enhanced degradation of mutant COMP by endogenous proteases, may also contribute to this phenomenon.

We now demonstrate for the first time that COMP interacts with collagen IX in a zinc-dependent manner, as observed previously for collagens I and II. The binding sites are present at the termini and two internal sites of the collagen IX molecule. Interestingly, inclusions in the ER have been found in all cell types in which COMP and collagen IX are coexpressed, namely, chondrocytes and fibrocartilaginous cells at the insertion site of ligaments (34, 53, 63). Tenocytes and ligament cells distant from the insertion sites express COMP but lack collagen IX, and no inclusions have been found in these cells (24, 34). Thus, we suspected that binding of mutant COMP to collagen IX could be the cause of the inclusions. Our results show that mutant COMP binds to collagen IX, albeit with an affinity similar to that of wild-type COMP. Thus, in the ER of chondrocytes and fibrocartilaginous cells, mutant COMP molecules might be recognized by a specific factor such as a chaperone, and subsequent binding of collagen IX to the COMP-chaperone complex might induce the formation of inclusions in the ER. A similar scenario is also likely for MED caused by mutations in collagen IX genes, which also show inclusions in chondrocytes (40).

Nonetheless, the binding of wild-type COMP to specific sites on collagen IX points to a physiological function of this interaction in the extracellular matrix. It was proposed that collagen IX, which is located on the surface of fibrils, represents a molecular bridge between fibrils and other matrix components and increases the stability of fibrillar network. This is in agreement with the late-onset osteoarthritis seen in collagen IX transgenic and deficient mice (44–47). The reduced binding of PSACH and MED mutant COMP to collagen IX might thus affect the stability of the fibrillar network.

The contribution of a disturbed collagen IX-COMP interaction to the molecular basis of the similar clinical phenotypes seen in MED patients with mutations in either collagen IX chains or COMP remains to be determined. We did not find pronounced differences between the COMP mutations that cause PSACH and MED, respectively, suggesting that additional factors contribute to the severity of these diseases.

Acknowledgments—We are grateful to S. Gößling for excellent technical assistance and Drs. F. Zaucke and R. Dinser for fruitful discussions. We thank Dr. M. Morgenlin for introducing D. F. N. into the methods for electron microscopy of macromolecules.

REFERENCES

1. International Working Group on Constitutional Diseases of Bone. (1998) Am. J. Med. Genet. 79, 576–582
2. Langer, L. O., Jr., Schaefer, G. B., and Wadsworth, D. T. (1993) Am. J. Med. Genet. 47, 772–781
3. Spranger, J. (1976) Clin. Orthop. 46–59
4. Hecht, J. T., Nelson, L. D., Crowder, E., Wang, Y., Elder, F. F., Harrison, W. R.
Effects of PSACH and MED Mutations in COMP

Francomano, C. A., Prange, C. K., Lennen, G. G., Deere, M., and Lawler, J. (1995) Nat. Genet. 10, 325–329

5. Engel, J., and Furthmayr, H. (1987) Methods Enzymol. 169, 61–73

6. Hedbom, E., Antonsson, P., Hjerpe, A., Aeschlimann, D., Paulsson, M., Rosa-Pimentel, E., Sommarin, Y., Wendel, M., Oldberg, A., and Heinegård, D. (1992) J. Biol. Chem. 267, 6132–6136

7. Malashkevich, V. N., Kammerer, R. A., Efimov, V. P., Schulthess, T., and Haüseltmann, H. J. (1998) Am. J. Med. Genet. 78, 199–206

8. Susic, S., Ahier, J., and Cole, W. G. (1997) J. Biol. Chem. 272, 12464–12468

9. Britten, P. J., and Mayne, R. (1988) in Structure and Function of Collagen Types (Mayne, R., and Burgeson, R., eds), pp. 195–221, Academic Press, Orlando, Fl.

10. Di Cesare, P. E., Hauser, N., Lehman, D., Pasumarti, S., and Paulsson, M. (1996) Br. J. Rheumatol. 35, 837–843

11. Di Cesare, P. E., Carlson, C. S., Stolleram, E. S., Chen, F. S., Leslie, M., and Perris, R. (1997) FEBS Lett. 412, 249–252

12. Engvall, E., Runswick, M., Aicher, W. K., Gay, R. E., Gay, S., and Heuselmann, H. J. (1998) Br. J. Rheumatol. 37, 721–728

13. Neckley, D. A., Buillargeron, L., and White, C. (1998) Arthritis Rheum. 41, 997–1006

14. Saxne, T., and Heinegård, D. (1992) Br. J. Rheumatol. 31, 593–598

15. Lehman, D., Saxne, T., and Heinegård, D. K. (1994) Ann. Rheum. Dis. 53, 8–13

16. Di Cesare, P. E., Carlson, C., Stolleram, E., Hauser, N., Tulli, H., and Pasumarti, S. (1996) J. Biol. Chem. 271, 14, 946–955

17. Neidhart, M., Hauser, N., Paulsson, M., Di Cesare, P. E., Michel, B. A., and Hausselmann, H. J. (1997) Br. J. Rheumatol. 36, 1115–1120

18. Kühne, S. A., Neidhart, M., Everson, M. P., Hantzschel, H., Fine, P. R., Gay, S., Hausselmann, H. J., and Gay, R. E. (1998) Rheumatol. Int. 18, 21–25

19. Rosenberg, K., Olsson, H., Mörgelin, M., and Heinegård, D. (1998) J. Biol. Chem. 273, 20397–20403

20. Colcke, D., Briggs, M. D., King, L. M., Rinoim, D. L., Wilcox, W. R., Lachman, R. S., and Knowlton, R. G. (1996) Ann. N. Y. Acad. Sci. 785, 188–194

21. Susie, S., McGrory, J., Ahier, J., and Cole, W. G. (1997) Clin. Genet. 51, 224–225

22. Ballas, R., Briggs, M. D., Cohn, D. H., Knowlton, R. G., Beighton, P. H., and Ramesar, R. S. (1997) Am. J. Med. Genet. 68, 396–400

23. Maddox, B. K., Keene, D. R., Sakai, L. Y., Charbonneau, N. L., Morris, N. P., Keene, D. R., Boswell, B. A., Sussman, M. D., Horton, W. A., Bachinger, H. P., and Hecht, J. T. (1997) J. Biol. Chem. 272, 30993–30997

24. Ferguson, H. L., Deere, M., Evans, R., Rotta, J., Hall, J. G., and Hecht, J. T. (1997) Am. J. Med. Genet. 78, 287–291

25. Briggs, M. D., Mortier, G. R., Cole, W. G., King, L. M., Golik, S. S., Bonaventure, J., Nuytinck, L., De Paep, A., Leroy, J. G., Biesecker, L., Lipson, M., Wilcox, W. R., Lachman, R. S., Rinoim, D. L., Knowlton, R. G., and Cohn, D. H. (1998) Am. J. Hum. Genet. 62, 311–319

26. Susie, S., Ahier, J., and Cole, W. G. (1998) Hum. Mutat. Suppl 1, S125–S127

27. Loughlin, J., Irven, C., Mustafa, Z., Briggs, M. D., Carr, A., Lynch, S. A., Knowlton, R. G., Cohn, D. H., and Sykes, B. (1998) Hum. Mutat. Suppl 1, S10–S17

28. Deere, M., Sanford, T., Ferguson, H. L., Daniels, K., and Hecht, J. T. (1998) Am. J. Med. Genet. 80, 510–513

29. Ito, K., Tani, K., Terada, N., Kitajima, Y., and Saito, M. (1999) Hum. Genet. 103, 633–638

30. Delot, E., King, L. M., Briggs, M. D., Wilcox, W. R., and Cohn, D. H. (1999) Hum. Mol. Genet. 8, 123–128

31. Deere, M., Sanford, T., Francomano, C. A., Daniels, K., and Hecht, J. T. (1999) Am. J. Med. Genet. 85, 287–291

32. Rizzo, R., Grandolfo, M., Godeas, C., Jones, K. W., and Vittor, F. (1995) J. Exp. Zool. 273, 82–86

33. Hecht, J. T., Montefusco, S. A., Cowen, W. J., Vertel, B., and Lawler, J. (1998) Matrix Biol. 17, 625–633

34. Smith, R. K., Zunino, L., Webbon, P. M., and Heinegård, D. (1999) Matrix Biol. 18, 265–277

35. Chen, H., Deere, M., Hecht, J. T., and Lawler, J. (2000) J. Biol. Chem. 275, 11412–11417
Mutations in Cartilage Oligomeric Matrix Protein Causing Pseudoachondroplasia and Multiple Epiphyseal Dysplasia Affect Binding of Calcium and Collagen I, II, and IX
Jochen Thur, Krisztina Rosenberg, D. Patric Nitsche, Tero Pihlajamaa, Leena Ala-Kokko, Dick Heinegård, Mats Paulsson and Patrik Maurer

J. Biol. Chem. 2001, 276:6083-6092.
doi: 10.1074/jbc.M009512200 originally published online November 17, 2000

Access the most updated version of this article at doi: 10.1074/jbc.M009512200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 64 references, 18 of which can be accessed free at http://www.jbc.org/content/276/9/6083.full.html#ref-list-1