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Type II Achondrogenesis-Hypochondrogenesis: Identification of Abnormal Type II Collagen

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Summary

We have extended the study of a mild case of type II achondrogenesis-hypochondrogenesis to include biochemical analyses of cartilage, bone, and the collagens produced by dermal fibroblasts. Type I collagen extracted from bone and types I and III collagen produced by dermal fibroblasts were normal, as was the hexosamine ratio of cartilage proteoglycans. Hyaline cartilage, however, contained approximately equal amounts of types I and II collagen and decreased amounts of type XI collagen. Unlike the normal SDS-PAGE mobility of coextracted types I and XI collagen chains, type II collagen chains exhibited retarded mobility. Two-dimensional SDS-PAGE revealed extensive overmodification of all type II cyanogen bromide peptides in a pattern consistent with heterozygosity for an abnormal proα1(II) chain which impaired the assembly and/or folding of type II collagen. This interpretation implies that dominant mutations of the COL2A1 gene may cause type II achondrogenesis-hypochondrogenesis. More generally, emerging data implicating defects of type II collagen in the type II achondrogenesis-hypochondrogenesis-spondyloepiphyseal dysplasia congenita spectrum and in the Kniest-Stickler syndrome spectrum suggest that diverse mutations of this gene may be associated with widely differing phenotypic outcome.

Introduction

In the preceding paper (Godfrey et al. 1988) we describe the clinical, radiographic, histologic, and ultrastructural abnormalities in a mild case of type II achondrogenesis-hypochondrogenesis fitting the classification criteria of Whitley-Gorlin prototype IV (Whitley and Gorlin 1983). Those findings are consonant with multiple other studies in this disease. Immunohistochemical studies using a monospecific monoclonal antibody against human type II (cartilage) collagen revealed a striking result: intense staining of small, rounded to oval structures within chondrocytes, strongly suggesting intracellular accumulation of this collagen type, probably in the distended cisternae of the rough endoplasmic reticulum observed in all chondrocytes by electron-microscopic studies. These observations raised the possibility of an abnormal type II collagen produced by, and accumulating within, chondrocytes.

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Type I collagen, a heterotrimer of two α1(I) and one α2(I) chains, is the most abundant protein in the body and the major collagen component of bone, tendon, skin, and many other tissues. In contrast, normal hyaline cartilage does not contain type I collagen but, instead, a distinctive set of cartilage-specific major and minor collagen types. These include type II (the major collagen), a homotrimer of α1(II) chains, and several so-called minor collagens (type IX, type X, and type XI [1α, 2α, 3α]) (Burgeson and Morris 1987). Identification of individual collagen types is facilitated by the characteristic SDS-PAGE mobilities of the constituent collagen α chains and is confirmed by cleavage of individual chains with cyanogen bromide. The resultant set of peptides (CB peptides) may be resolved by SDS-PAGE or chromatography and may serve as a fingerprint for specific collagen chains (Click and Bornstein 1970; Epstein et al. 1971; Miller and Lunde 1973).

The studies of Horton et al. (1985*a*, 1985*b*), Eyre et al. (1986), and Murray et al. (1987) have provided collagen compositional data on type II achondrogenesis-hypochondrogenesis. Horton et al. (1985*a*) found CB peptides for both types I and II collagen in the cartilage of one patient and confirmed this result in four subse-

quent cases (Horton et al. 1985*b*). In a more detailed study of a moderately severe case, Eyre et al. (1986) found type I collagen together with small amounts of types IX and XI collagen in hyaline cartilage; type II collagen was undetectable. The ratio of galactosamine to glucosamine of extracted cartilage proteoglycans was normal. These findings suggested that chondrocyte differentiation had occurred, but without the concomitant expression of type II collagen. In a recent contribution, Murray et al. (1987) confirmed the absence of type II collagen in cartilage from a relatively severe patient, but they observed the admixture of types I and II collagen in three less severe cases. Notably, the type II collagen exhibited delayed electrophoretic mobility, consistent with overmodification of α1(II) chains.

In the present communication, we report biochemical analyses of cartilage, bone, and the collagens produced by dermal fibroblasts. The major findings include the presence of type I collagen in cartilage together with an abnormal type II collagen exhibiting overmodification in a pattern consistent with the presence of a single abnormal allele of the COL2A1 gene. We interpret these data to indicate that a heterozygous mutation of the COL2A1 gene is the cause of this patient's disease.

Material and Methods

Tissue Sources

Patient and age-matched control material was obtained as described elsewhere (Godfrey et al. 1988) and stored at −120 C. Type I collagen was extracted from human placentas by standard techniques.

Collagen Extraction from Cartilage

Samples of patient and control femoral head cartilage were carefully dissected free of soft tissue, perichondrium, bone, and growth plate and diced into 1-mm^2 pieces. There were no epiphyseal ossification centers in either sample. The samples were homogenized in water, lyophilized, and extracted at 4 C in 4 M guanidine HCl, 50 mM Tris-HCl (pH 7.4) for 2 days. Following centrifugation (1.05×10^6 $g \cdot$ min), the supernatant was dialyzed against dilute acetic acid and lyophilized (proteoglycan fraction). The precipitate was digested in 1 M acetic acid containing 100 μg of porcine pepsin (Boehringer)/ml for 16 h at 4 C. The resultant cloudy solution was clarified to yield an insoluble fraction, and the viscous, slightly turbid supernate was subjected to ultracentrifugation (4.2×10^6 $g \cdot$ min). The precipitate, enriched for molecules containing 1α, 2α, and 3α chains (type XI collagen fraction), was dissolved in 1 M NaCl, 50 mM Tris (pH 7.5), and the pH was adjusted to 8.0 with Tris base and stirred at 4 C for 3 days; thereafter, it was dialyzed against dilute acetic acid and lyophilized. The clear supernatant following ultracentrifugation was adjusted to 2 M by adding solid NaCl and was stirred for 1 h at 4 C, and the precipitate was collected by centrifugation (1.05×10^6 $g \cdot$ min). The precipitate was redissolved in 1 M acetic acid and reprecipitated with 2 M NaCl as before. This step was repeated a total of three times. The final precipitate (type II collagen fraction) was processed as described above. All fractions were stored at −120 C.

Bone Collagen

Patient and control femurs were carefully cleaned of all soft tissue and decalcified for 90 days at 4 C in 50 mM Tris, 0.2 M EDTA (pH 7.5). Collagen was extracted as described above.

Fibroblast Collagens

^{3}H-proline-labeled collagens were obtained from patient and control fibroblasts by using a slight modification of a published procedure (Barsh and Byers 1981).

Collagen Thermal Denaturation (Melting) Temperature

Melting temperatures were determined from 25 C to 42 C (at 1-degree-C increments) in parallel for collagens from control and patient cartilage and type I collagen from placenta, by using the modified version (Steinmann et al. 1984) of the proteolytic method described by Bruckner and Prockop (1981).

Cyanogen Bromide (CNBr) Cleavage of Collagen

Collagen samples were dissolved at 1 mg/ml in 70% formic acid containing 50 mg resublimated CNBr (Eastman Kodak)/ml and were cleaved according to a method described elsewhere (Epstein et al. 1971).

SDS-PAGE

Discontinuous SDS-PAGE gels were prepared as described by Laemmli (1970). Whole collagen chains were analyzed in 5% gels and cyanogen bromide peptides were analyzed in 12% gels beneath 3% stacking gels. For two-dimensional (2D) electrophoresis, the collagen chains were separated by SDS–5% PAGE gels, and lanes of interest were cut out and treated with CNBr by using a slight modification of a described technique (Barsh et al. 1981; Steinmann et al. 1984; V. H. Rao, personal communication), inserted above, and resolved by SDS–10% PAGE in the second dimension. Proteins

were visualized with 0.2% Coomassie blue and were photographed by using Kodak electrophoresis duplicating paper. Radiolabeled collagens were detected by exposing gels to Kodak X-Omat film.

Amino Acid and Hexosamine Analysis

Analysis of amino acids and hexosamines was performed by hydrolysis and derivatization using phenylisothiocyanate (PITC) in a PICO-TAG system according to methods supplied by the manufacturer (Waters, Milford, MA), as described elsewhere (Morris et al. 1986). Response factors were determined by using amino acids standards (CH Collagen Standard; Pierce, Rockford, IL) and purified glucosamine and galactosamine standards (Applied Science, State College, PA). Proteoglycan samples were analyzed as described elsewhere (Eyre et al. 1986).

Results

Cartilage: Gross Appearance and Consistency

The cartilage from the patient appeared similar to that from the control sample but had a softer, more spongy texture and was somewhat fibrous and more difficult to cut into regular-sized fragments. Much less effort was needed to homogenize the patient cartilage than the control cartilage.

Cartilage and Collagen Extractability

Femoral head cartilage samples were extracted as described above, and the resultant fractions (including residues not solubilized by pepsin) were dialyzed, lyophized, and weighed. The results are recorded in table 1, which indicates the percentages of each fraction on the basis of both wet and dry weights.

The hydroxyproline content of the proteoglycan fractions was determined as a measure of collagen extractability. Expressed per milligram of proteoglycan fraction, the values were 22.0 μg for the control and 7.3 μg for the patient.

Hexosamine Content and Proteoglycans

Figure 1 depicts the quantitation of galactosamine and glucosamine, in picomoles per microgram of proteoglycan fraction, determined at various hydrolysis time points. The total amount of each hexosamine was determined by least-squares extrapolation to zero time of hydrolysis. Galactosamine-to-glucosamine ratios computed with the extrapolated values were 6.7 for control and 6.0 for patient proteoglycans.

Electrophoresis of Cartilage Collagens

SDS–5% PAGE of patient and control type II collagen fractions is shown in figure 2 and compared with type I collagen from human placenta. The single, intensely staining band in lane B is the α1(II) chain of control type II collagen which comigrates with the α1(I)

Table 1

Fractionation of Control and Patient Hyaline Cartilage

	% of Total Weight			
	Control Cartilage[a]		Patient Cartilage[b]	
Fractions Extracted	Wet	Dry	Wet	Dry
Collagen[c]	9.54	36.82	4.14	53.19
Proteoglycans	9.02	34.83	3.90	40.43
Insoluble	4.77	18.41	.62	6.38

[a] Control femoral head cartilage: wet weight 776 mg, dry weight 201 mg (26% of wet weight).

[b] Patient femoral head cartilage: wet weight 487 mg, dry weight 47 mg (10% of wet weight).

[c] Collagen values represent sums of the types II and XI collagen fractions.

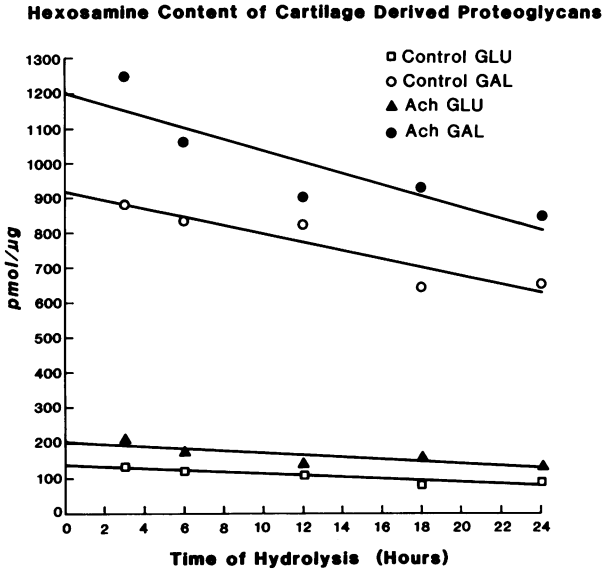

Figure 1 Galactosamine and glucosamine content per microgram proteoglycan extracted from hyaline cartilage as a function of hydrolysis time. ● = Galactosamine from patient; ○ = galactosamine from control; ▲ = glucosamine from patient; □ = glucosamine from control. Lines represent the least-squares analysis of the data points.

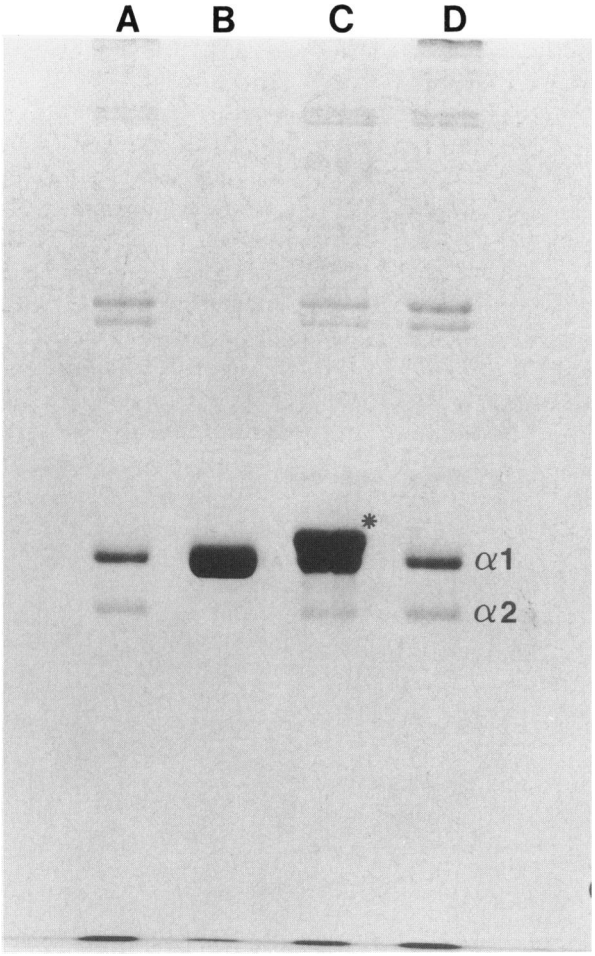

Figure 2 Single-dimension slab-gel electrophoresis (SDS–5% PAGE) of collagens. Lanes A and D, Normal type I collagen from placenta. The identity of each collagen chain is indicated. Lane B, Type II collagen from control hyaline cartilage, deliberately overloaded for comparison. Lane C, Collagens from the cartilage of the patient. This lane is also overloaded to emphasize the α2(I) chain. The asterisk (*) denotes a band migrating more slowly than α1 chains of either type I or type II collagen.

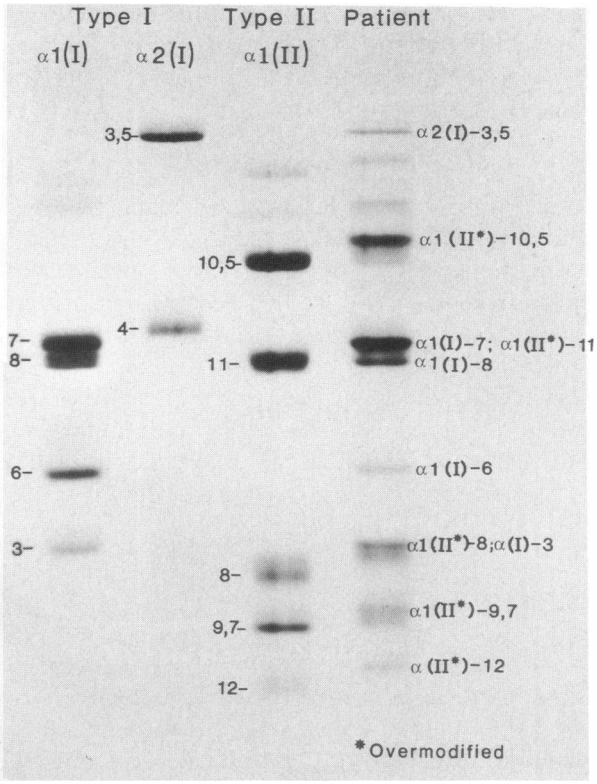

Figure 3 Slab-gel electrophoresis (SDS–12% PAGE) of cyanogen bromide (CB) peptides from different collagens. The α1(I) and α2(I) CB peptides are shown in the two left lanes. The peptides derived from normal type II collagen and from patient cartilage–derived collagens are shown at right. The numbers to the left of the control types I and II collagen lanes identify the CB peptides. The designations to the right of the patient lane identify the CB peptides of types I and II collagen, and the slower-migrating (overmodified) type II collagen CB peptides are indicated by an asterisk (*). Note that all patient type II–derived CB peptides from CB 12 to uncleaved, high-molecular-weight materials are electrophoretically delayed as compared with the control.

chain of type I collagen in SDS-PAGE. The materials in lane C are the collagens extracted from patient cartilage. There is a fast-migrating band which comigrates with the α2(I) chain of type I collagen. This lane also contains a band that comigrates with the α1 chain of both types I and II collagen. In addition, there is a band of more slowly migrating material just above the α1(I) chain (denoted by an asterisk [*]). This band represents overmodified type II collagen (see below). By visual inspection of the original gel, there appeared to be approximately a 1:1 ratio of types I and II collagen. Finally, slower-migrating dimeric collagen chains which resembled those observed for type I collagen were apparent, and higher-molecular-weight trimeric chains resembling type III collagen were observed.

To determine collagen types, the cartilage collagens were subjected to CNBr digestion and resolved by SDS–12% PAGE. CB peptides derived from purified normal human fibroblast α1(I) and α2(I) chains (a gift from Dr. Mary Wirtz) and control type II collagen were coelectrophoresed to serve as controls (fig. 3). The cartilage collagens from the patient contained CB peptides of both types I and II collagen, indicating the admixture of these different collagens. The CB peptides de-

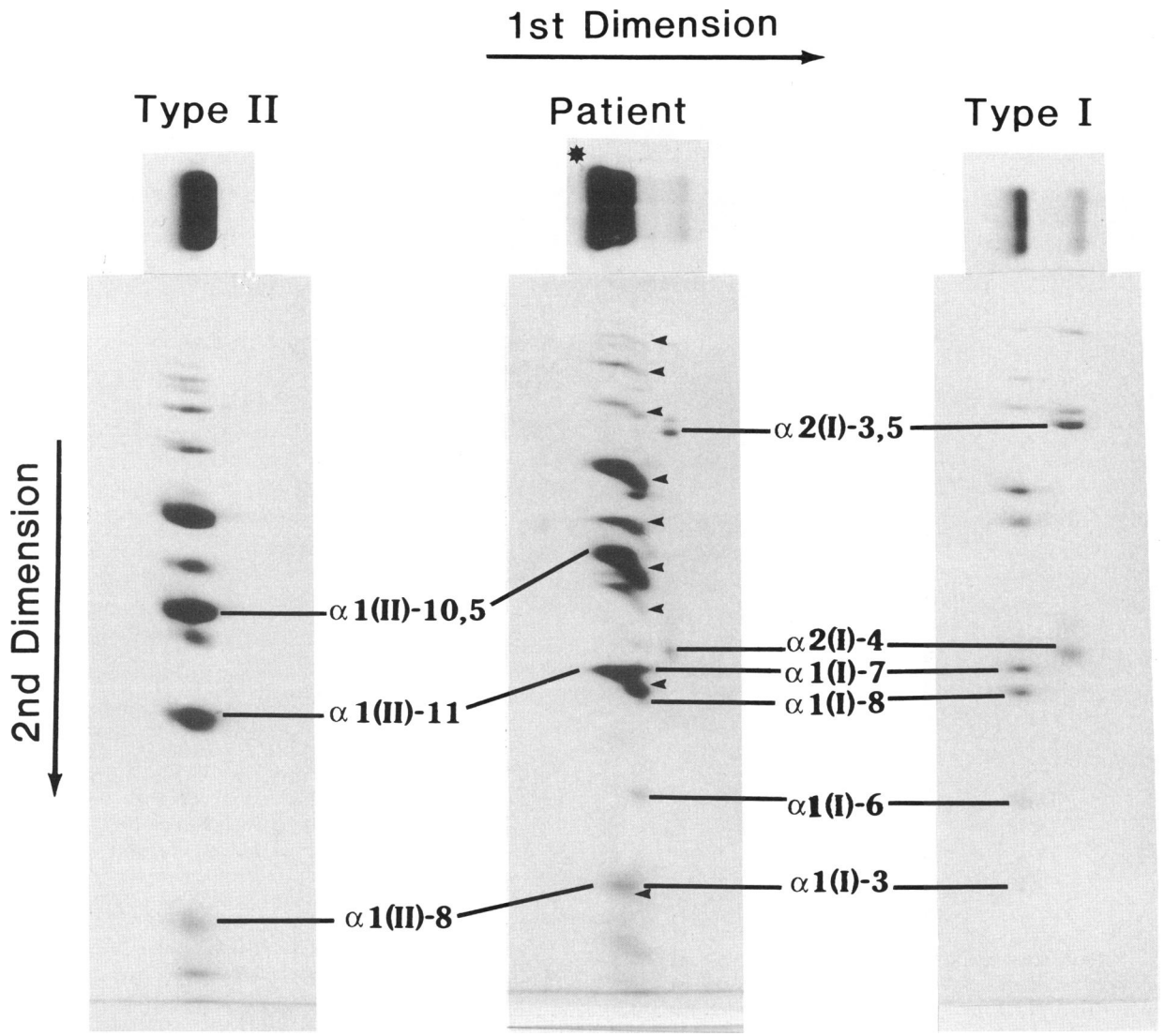

Figure 4 2D electrophoresis of collagen from normal and patient femoral hyaline cartilage, and type I collagen from human placenta. The first-dimension bands are included for orientation purposes at the top of the figure, and the asterisk (*) identifies the overmodified type II collagen. Bands in the patient cartilage (*middle*), which correspond to CB peptides in control type I collagen (*right*), are identified to the right of the patient lane. Likewise, bands from the patient cartilage (*middle*), which correspond to CB peptides in normal type II collagen (*left*), are labeled to the left of the patient lane. The overmodified type II collagen CB peptides of the patient have a "tail" (small arrowheads), which indicates that a small portion of the peptides exhibit normal electrophoretic mobility in both dimensions.

rived from type I collagen appear to migrate as sharp bands and identically with control peptides (compare the α1(I) CB 7, 8, 6, and 3 and α2(I) CB 3,5 of the control with the patient's CB peptide profile). In contrast, all of the patient's type II CB peptides (identified by an asterisk[*]) migrate more slowly than their normal counterparts. This observation strongly suggests that these peptides are overmodified, i.e., contain increased contents of hydroxylysine and glycosylated hydroxylysines. Close inspection of these peptides reveals that the great majority of stainable material is delayed in migration, but a weakly staining smear extends below the major band to the electrophoretic position of the control type II collagen CB peptides. This is particularly noticeable for CB 10,5 and CB 9,7. This faint smearing of the patient's peptides suggests that

a small fraction of each CB peptide is more normal in mobility and escapes overmodification.

To further evaluate the patient's cartilage collagens, 2D SDS-PAGE was performed. Figure 4 is a composite of 2D gels of control types I and II collagen and of cartilage-derived collagens from the patient. This analysis clearly demonstrates that the slow-migrating band (indicated by an asterisk [*] in the inset one-dimensional gel above the 2D profile) is indeed type II collagen, and all type II peptides appear overmodified. As before, the peptides derived from the chains of type I collagen exhibit normal electrophoretic mobility.

The most striking feature in the 2D gel analysis of the patient material is the presence of a "tail" extending from each of the α1(II) CB peptide bands (fig. 4), small arrowheads). These tails represent peptide materials that have migrated faster in both SDS-PAGE dimensions and are seen for all type II–derived peptides. When a freshly stained gel is examined, the type II bands stain much more intensely than the corresponding tails, but the proportion of stainable materials (main band vs. tail) seems similar for all peptides. We estimate that 80%–90% of the stainable material is in the band while 10%–20% of the stainable material is found in the tail. Notably, the tails are positioned toward the point where the normal type II CB peptides migrate and appears to represent a gradient from normal mobility to delayed mobility. The tails appear to represent the 2D expression of the faint smearing observed beneath the type II CB peptides shown in figure 3.

Analysis of the cartilage collagen fraction enriched for type XI collagen revealed diminished amounts of this minor collagen in the patient sample as compared with the control sample. The SDS-PAGE mobility of the observed 1α, 2α, and 3α chains, however, were comparable to that of the control sample, and the relative amounts of each of these chains appeared normal (data not shown). In addition, no differences in SDS-PAGE mobility were observed between radioactively labeled normal and patient fibroblast-derived collagens, and patient type I collagen, solubilized from decalcified bone, was comparable to that of the control sample (data not shown).

Amino Acid Analyses

Amino acid analyses were performed on control types I and II collagens and on the patient cartilage-derived collagens (containing a mixture of types I and II); the partial amino acid compositions determined are recorded in table 2. The column labeled "1:1 Mixture" depicts a theoretical calculation for an equal mixture of normal types I and II collagen. The patient cartilage-derived collagens contain slightly more hydroxylysine than does pure type II collagen and contains five more hydroxylysine and five fewer lysine residues than does the calculated 1:1 mixture of normal types I and II collagen. Assuming normal hydroxylation of type I collagen and a 1:1 distribution of types I and II collagen, we calculate that as many as 10 additional lysines have been hydroxylated in the abnormal type II collagen. These data imply a 73% hydroxylation of lysine in the abnormal type II, in contrast to the 50% observed in normal type II, and directly confirm the overmodification suggested by SDS-PAGE. Proline and hydroxyproline content is within experimental (<5% difference) error of the expected values.

Table 2

Partial Amino Acid Content of Normal and Patient Collagens

	Residues/1,000			
	Type I[a]	Type II[b]	Patient[c]	1:1 Mixture[d]
Hydroxyproline	98	105	106	102
Proline	120	101	115	110
Hydroxylysine	14	22	23	18
Lysine.....	28	22	20	25
Total (Lys + Hylys)	42	44	43	43

Note.—Values are uncorrected partial amino acid content in residues/1,000. Control types I and II collagen represent means of duplicate analyses; the patient material is from a single analysis.

[a] Normal human type I collagen (placenta).

[b] Normal human type II collagen (femoral hyaline cartilage).

[c] Patient cartilage–derived collagens (femoral hyaline cartilage).

[d] Expected residues/1,000 for a 1:1 mixture of normal human types I and II collagen.

Melting Temperature

The melting temperature of purified control collagens and cartilage-derived collagen from the patient were simultaneously determined. Smooth melting curves were obtained for all samples. The midpoint of the melting transition (T_m) for control type II collagen was 40–41 C and the T_m for control type I collagen was 41–42 C, while the T_m for the cartilage-derived collagens of the patient (containing both type I and overmodified type II collagens) was 41–42 C (data not shown).

Discussion

The physical consistency of the patient's femoral head cartilage was more pliant and spongy than that of the control sample. Comparisons of wet and dry weights (Table 1) suggest that the patient's cartilage was excessively hydrated (edematous) with a decreased accumulation of collagen and proteoglycans. On a dry-weight basis, however, there was relatively more collagen than proteoglycan. These data may reflect the presence of the extensive fibrovascular tissue (see Godfrey et al. 1988) and/or indicate that proteoglycans are not appropriately entrapped by—and therefore escape from—the edematous cartilage matrix. The insoluble fraction of the patient's cartilage was substantially less than that of the control sample. This apparent increased solubility, however, was not due to increased extractability of collagen(s), since hydroxyproline assays of the 4 M guanidine extracts were lower in the patient than in the control sample. On the basis of hydroxyproline extraction, there is no evidence for defective cross-linking of collagens.

Hexosamine assays of proteoglycan fractions of the patient and control samples were comparable (fig. 1) and yielded normal ratios of galactosamine to glucosamine. These data suggest that chondrocyte differentiation as judged by the production of cartilage-specific proteoglycan has occurred. These findings are similar to previous data in a more severe case (Eyre et al. 1986).

A significant finding is type I collagen in hyaline cartilage, confirming previous data (Horton 1984; Horton et al. 1985*a*, 1985*b*, 1987; Eyre et al. 1986; Murray et al. 1987). Undoubtedly, some proportion of this type I is derived from the prominent fibrovascular canals, but, as discussed in the preceding paper (Godfrey et al. 1988), it may also be derived from the cartilage matrix proper. It is noteworthy that the types I and III collagens coisolated with type II collagen from the patient's cartilage have normal SDS-PAGE mobilities and normal CB peptide profiles. Similarly, the types I and III collagens produced by cultured dermal fibroblasts and the type I collagen directly extracted from bone were normal by these criteria.

The major finding in the present case is abnormal type II collagen. This collagen and derived CNBr peptides exhibited delayed migration in one-dimensional SDS-PAGE (figs. 2, 3) and in 2D SDS-PAGE (fig. 4), results consistent with overmodification. Overmodification describes increased hydroxylysine and glycosylated hydroxylysines (gal-Hylys and glu-gal-Hylys) in α chains and derived peptides (Steinmann et al. 1986; Bateman et al. 1987*a*). Collagen chains are initially synthesized as larger precursors, proα chains, which differ from α chains by virtue of both amino and carboxyl extension peptides (propeptides). Assembly of collagen trimers is mediated by interaction of the carboxyl propeptides and is followed by folding of the triple helix, which proceeds unidirectionally from carboxyl to amino terminus (Bachinger et al. 1981) to yield the precursor molecule, procollagen. Modifying enzymes, such as lysyl hydroxylase and the sugar transferases, utilize denatured proα chains as substrates but cannot modify native (folded) procollagen molecules (Kivirikko and Myllyla 1984). Modification, therefore, is a (normally incomplete) kinetic process that occurs during synthesis and assembly and is terminated by folding of the triple helix. Delayed assembly and/or folding permits additional modification reactions to occur and leads to overmodification. Variable overmodification (for type I collagen) has been frequently observed in osteogenesis imperfecta and is due to mutations that apparently impede collagen folding. There is now good evidence that overmodification begins at or near the rate-limiting site (mutation) and results in overmodification of all three proα chains distal (i.e., in an amino-terminal direction) to this site (Bateman et al. 1984, 1987*a*, 1987*b*; Steinmann et al. 1984; Bonadio and Byers 1985; Vogel et al. 1987; Byers et al. 1988). In the present case, since all CB peptides are overmodified, the entire α1(II) chain is overmodified.

An additional abnormality of virtually all overmodified osteogenesis imperfecta mutant collagens is decreased thermal denaturation (melting) temperatures (Bonadio and Byers 1985) and occurs presumably because many helical region mutations destabilize the molecule. One notable exception has been found in which the mutation occurs at the extreme carboxyl end of the molecule (Wenstrup et al. 1988).

Of present interest is the possibility of a mutation in one allele of the COL2A1 gene. If equivalent transcription, translation, and random selection of chains during assembly are assumed, trimers would be produced in eight possible combinations, of which seven would contain one, two, or three mutant chains (see Stolle et al. 1985). Therefore, if one or more mutant chains delayed assembly and/or folding, nearly 90% of the resultant molecules would exhibit overmodification and retarded SDS-PAGE migration, and only about 10% would escape overmodification and demonstrate normal SDS-PAGE mobility. As demonstrated in figure 4, each type II CNBr peptide exhibits a retarded main band representing about 80%–90% and a faintly stain-

ing "tail" of more normal mobility representing about 10%–20% of the total stainable material.

Although present in reduced amounts, the chains of type XI collagen (1α, 2α, and 3α) demonstrated normal electrophoretic mobility and relative amount. Since type XI is a minor, cartilage-specific collagen which is uniformly distributed in hyaline cartilage (Burgeson and Hollister 1979) and is a biosynthetic product of chondrocytes (Eyre and Wu 1987; Morris and Bachinger 1987), we presume that the same cells that produced overmodified type II also produced normally modified type XI. This reasonable assumption implies a specific molecular defect of type II collagen, rather than a generalized defect in chondrocyte collagen biogenesis.

We interpret these data to indicate heterozygosity for a mutant allele of the COL2A1 gene, which produced a structurally abnormal procollagen chain, which, in turn, caused impaired assembly and/or folding and subsequent overmodification. Because the entire α1(II) chain is overmodified, we suspect that the protein defect resides at or near the carboxyl terminus or within the carboxyl propeptide of the mutant proα1(II) chain. The normal melting temperature of the overmodified type II molecule found here also argues that the protein defect is in this region. A proposed pathogenesis for this disease is shown in the Appendix and is consistent with data from the present and previous studies. The central pathogenetic mechanism would appear to be absent or diminished matrix deposition (expression) of type II collagen, as first suggested by Eyre et al. (1986). By analogy, this formulation is also consonant with the known pathophysiologic consequences of mutant types I and III collagens in osteogenesis imperfecta and Ehlers-Danlos syndrome type IV, respectively (e.g., see Byers et al. 1979; Clark et al. 1980; Barsh and Byers 1981; Williams and Prockop 1983; Prockop 1984; Steinmann et al. 1984; Holbrook and Byers 1987). The proposed pathogenesis is not consistent with recessive inheritance. However, most cases of this disease have been sporadic and the absence of parental consanguinity is noteworthy (Maroteaux et al. 1983; Borochowitz et al. 1986), favoring new dominant mutations. Familial recurrences (Whitley and Gorlin 1983) may reflect true recessive inheritance or, possibly, gonadal mosaicism similar to that proposed for recurrent type II (lethal) osteogenesis imperfecta (Byers et al. 1988).

We postulate that heterozygous mutations of the COL2A1 gene cause most cases of type II achondrogenesis-hypochondrogenesis. It seems likely that variations in the specific mutations—and in particular, their consequences for molecular assembly—will account for the observed clinical heterogeneity. The most severe molecular defects presumably prevent collagen assembly and/or folding and therefore secretion, and the resultant cartilage matrix contains little or no type II collagen, as observed by Eyre et al. (1986) and Murray et al. (1987). The presence of somewhat less severe defects (exemplified by the present case and those of Murray et al. [1987]) permits appreciable folding (with extensive overmodification), secretion, and accumulation of type II molecules in cartilage. At present, there would appear to be an inverse relationship between phenotypic severity and apparent amount of type II collagen accumulating in cartilage matrix.

Recently, Spranger (1985) has introduced the concept of "bone dysplasia families" as exemplified by the similar pattern of radiographically demonstrated skeletal abnormalities (dysostosis multiplex) produced by a variety of lysosomal storage diseases. The concept postulates that the developing skeleton reacts in a stereotypic fashion to heterogeneous disturbances of a single metabolic pathway. Spranger defines one such family as consisting of type II achondrogenesis, hypochondrogenesis, and spondyloepiphyseal dysplasia congenita (SEDc), which form a spectrum of decreasing severity. SEDc is a diverse group of typically nonlethal dwarfing disorders, usually inherited in a dominant fashion; the more severe variants of this disorder overlap with hypochondrogenesis (Maroteaux 1976; Borochowitz et al. 1986). Type II collagen has now been found to be variably overmodified within the helical region in a number of SEDc cases, strongly suggesting diverse molecular defects of this protein (Murray and Rimoin 1988). Yet another bone dysplasia family is the Kniest-Stickler syndrome spectrum. Direct evidence for the involvement of type II collagen in this family has recently emerged. Poole et al. (1988) have obtained evidence indicating abnormal removal of the carboxyl propeptide of type II collagen in four cases of Kniest dysplasia. Francomano et al. (1987), using the candidate gene approach, have linked the type II collagen gene to Stickler syndrome with a lod score of >3 at $\theta = 0$ in a large family.

These various considerations suggest that mutations of the type II collagen gene may be associated with an enormous range of phenotypic abnormalities, from lethal short-limbed dwarfism to a relatively normal phenotype with myopia and early arthritis. Presumably, the specific mutational event and the diverse consequences for the structure and function of the type II molecule will account for the differences in phenotype. This notion is in accord with the emerging information

relating molecular defects of the type I collagen molecule to the phenotypically heterogeneous diseases comprising osteogenesis imperfecta and some of the Ehlers-Danlos syndrome variants (Prockop and Kuivaniemi 1986).

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Appendix

Proposed Pathogenesis

1. Heterozygous mutation of COL2A1 gene
2. Synthesis of a structurally abnormal proα1(II) chain
3. Impaired assembly and/or folding of type II molecules
   a. Overmodification of almost all type II molecules
   b. Intracellular accumulation within dilated RER
   c. Probable increased intracellular degradation
   d. Inappropriate synthesis and secretion of types I/III collagen(?)
4. Decreased secretion of type II collagen
5. Matrix: relative hypercellularity with deficient matrix accumulation; hypofibrillar; perivascular fibrosis (? secondary effect)
6. Proportionately smaller cartilagenous structures (femoral head, etc.)
7. Deficient endochondral ossification
8. Dwarfism, pulmonary hypoplasia, early respiratory death

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