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Molecular cloning of the human α2(IX) collagen cDNA and assignment of the human COL9A2 gene to chromosome 1

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Type IX collagen, a heterotrimer of α1(IX), α2(IX) and α3(IX) chains, is a cartilage-specific fibril-associated collagen. In the process of characterizing genomic clones for the mouse α2(IX) collagen gene four pairs of oligonucleotide primers designed for amplification of murine exon sequences were also utilized to construct cDNA clones for human α2(IX) collagen spanning > 90% of the coding region. The amino acid and nucleotide sequence identities between human and chick are 78% and 71%, respectively. Localization of the COL9A2 gene to human chromosome 1 was subsequently performed using a panel of DNAs from human/rodent somatic cell hybrids.

Cartilage; Collagen; Human chromosome 1; mRNA; Polymerase chain reaction

1. INTRODUCTION

The collagen protein family now consists of at least 18 members with a minimum of 30 genes coding for their constituent α-chains [1,2]. A majority of these genes have been cloned and mapped for at least one mammalian species. Type IX collagen, a heterotrimer of α1(IX), α2(IX), and α3(IX) chains, is a minor component of collagen fibrils in hyaline cartilage [3]. It associates laterally on the fibril surface [4] and is stabilized through covalent cross-links to type II collagen [5,6]. The α2 chain of type IX collagen contains a proteoglycan side chain [7]. Through these properties type IX collagen is believed to regulate the diameter of cartilage collagen fibrils [8] and to mediate the interactions between cartilage collagens and proteoglycans [9].

No known mutations exist in type IX collagen. However, the molecular basis of most of the hereditary diseases affecting cartilage (chondrodysplasias) remains unknown [10]. Before the role of type IX collagen in chondrodysplasias and other diseases of cartilage can be determined molecular probes for the three constituent chains of human type IX collagen must be obtained. Full length cDNA clones have been reported for human α1(IX) collagen [11,12], but none exist for human α2(IX) and α3(IX) collagen chains.

We have recently used RT–PCR (reverse transcription–polymerase chain reaction) technology to construct short cDNA clones for mouse α1(IX) and α2(IX) collagen mRNAs [13,14] and continued by isolating the entire mouse α2(IX) collagen gene (Perälä & Vuorio, manuscript in preparation). Here we report on the use of the mouse *Col9a2* sequence information for amplification and cloning of essentially the full coding sequence of the human α2(IX) collagen mRNA. The chromosomal localization of the human COL9A2 was subsequently performed using a panel of DNAs from human/rodent somatic cell hybrids.

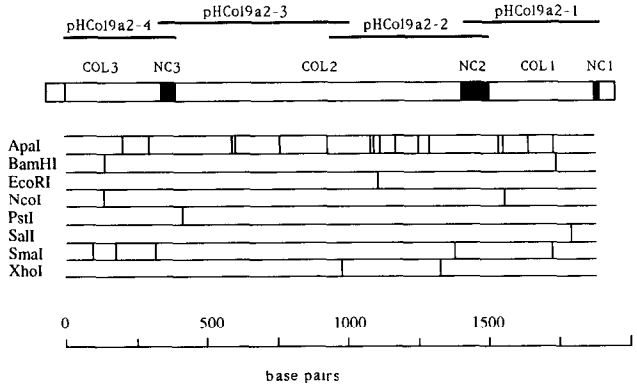

Fig. 1. The restriction map and coding domains of the human COL9A2 cDNA assembled from overlapping clones pHCol9a2–1, -2, -3, and -4.

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Abbreviations: PCR, polymerase chain reaction; RT–PCR, reverse transcription–polymerase chain reaction.

The sequence described in this paper has been submitted in GenBank/EMBL Data Library under the accession number M95610.

2. MATERIALS AND METHODS

2.1. *cDNA cloning of human α2(IX) collagen mRNA by RT–PCR*

Random hexamers and oligo(dT) were used to prime reverse transcription of total RNA from human fetal cartilage. Specific cDNA fragments were amplified in four PCR reactions using 18-mer oligonucleotides based on the mouse sequence as primers. The reactions were cycled through denaturation at 94°C for 1 min, annealing at 50°C for 2 min, and extension at 72°C for 2 min. The cDNA fragments were purified on agarose gels and cloned by blunt end ligation into the *Eco*RV site of Bluescript KS$^-$ using standard techniques described elsewhere [13–15]. The cloned fragments and derived subclones and sequenced using the Sanger dideoxy method (Sequenase™ reagent kit). The amplification strategy used to obtain overlapping PCR fragments is shown in Fig. 1.

2.2. *Chromosomal assignment*

A mapping panel composed of genomic DNAs of 18 human-rodent somatic hybrid cell lines plus human, hamster and mouse control DNA was obtained from Coriell Institute, Camden, NJ [16–19]. 10 μg of human and mouse DNA was digested to completion with *Eco*RI. The restriction fragments were separated in 0.8% agarose gel and

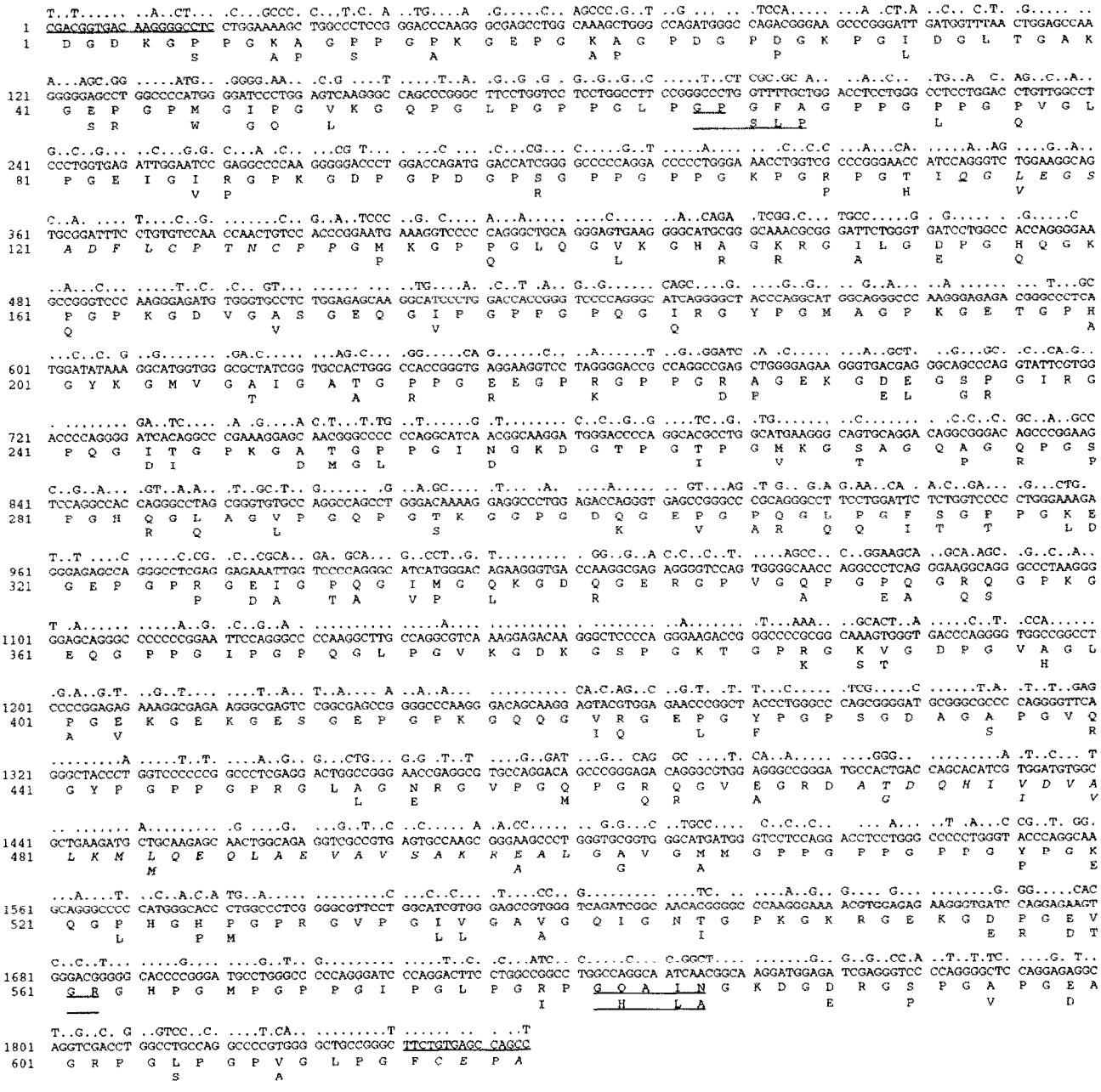

Fig. 2. The nucleotide sequence of the human COL9A2 cDNA assembled from overlapping clones pHCol9a2–1, -2, -3, and -4. Both the nucleotide sequence (second line) and the deduced amino acid sequence (third line) are compared with the corresponding chick sequences (first and fourth lines, respectively). Chick nucleotides and amino acids are only shown when different from human. Identical nucleotides in the chick are shown with dot. The nucleotide sequence corresponding to the oligonucleotides used for amplification of 5′- and 3′-terminal sequences are underlined to remind that these represent mouse sequences. The amino acids in the noncollagenous NC3, NC2 and NC1 domains are italicised, and the short discontinuities in the triple-helical Gly-X-Y structure underlined. Both nucleotides and amino acids are numbered on the left.

transferred onto a nylon membrane (Zetaprobe, BioRad). The membranes were hybridized with cDNA probes labelled with [α-^{32}P]dCTP in 1 M NaCl/1% SDS/10% dextran sulphate with 25 mg/ml denatured herring sperm DNA at 65°C overnight. The membranes were washed at high stringency and autoradiographed with intensifying screens for 7 days at −80°C.

3. RESULTS

3.1. *Nucleotide sequence of the human α2(IX) collagen cDNA*

The amplification strategy of the human α2(IX) collagen mRNA is shown in Fig. 1. Four pairs of oligonucleotide primers were designed for RT–PCR based on the sequence of the murine *Col9a2* gene. The use of mouse-specific oligonucleotides was considered reasonable since an approximately 90% similarity has been observed in collagen sequences between mouse and man [20]. The same oligonucleotides were found to prime the amplification of both mouse and human α2(IX) collagen cDNA.

The nucleotide sequence and deduced amino acid sequence of the human α2(IX) collagen cDNA shown in Fig. 2 was obtained by sequencing of the cloned PCR fragments and subclones derived from these. The domain structure of the human α2(IX) collagen chain and the restriction map of the corresponding cDNA are shown in Fig. 1.

3.2. *Chromosomal assignment*

For chromosomal assignment of the human COL9A2 gene the labeled cDNA probe was hybridized to a mapping panel composed of genomic DNAs of 18 human–rodent somatic hybrid cell lines plus human, hamster and mouse control DNA [16–19]. *Eco*RI was used for digestion of the DNAs since this enzyme produced fragments of different size from human and mouse α2(IX) collagen genes. Among the hybrid cell lines examined

Table I

Assignment of *COL9A2*

	human chromosome																							
DNA #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	Y	*COL9A2*
NA09925	+	+	−	+	+	+	+	+	−	−	(+)	+	(+)	+	+	+	+	+	+	+	−	+	−	−	+
NA09926	+	+	+	+	(+)	+	+	+	−	+	−	(+)	+	+	+	(+)	+	+	+	+	(+)	(+)	(+)	−	+
NA09927	+	+	+	+	−	+	+	+	−	+	−	−	+	+	+	−	+	+	+	+	−	−	−	−	+
NA09928	−	+	+	−	+	+	−	+	−	(+)	−	−	(+)	+	+	−	+	−	+	−	+	+	−	+	−
NA09929	−	−	+	+	−	+	(+)	+	−	−	+	+	−	+	(+)	−	+	−	(+)	+	−	−	(+)	−	−
NA09930A	−	+	+	(+)	+	−	+	(+)	−	−	(+)	+	+	+	+	−	+	+	−	+	+	+	(+)	(+)	−
NA09931	−	−	−	−	+	−	+	−	−	+	−	+	−	+	−	−	+	−	−	+	+	−	−	+	−
NA09932	−	−	−	+	+	+	−	+	−	(+)	+	+	−	−	−	−	+	−	(+)	−	+	−	−	−	−
NA09933	+	−	+	+	+	+	+	+	−	(+)	−	+	+	+	+	−	+	+	+	+	+	+	−	+	+
NA09934	−	+	−	−	+	+	(+)	+	−	−	+	+	−	(+)	+	−	+	+	−	+	+	(+)	−	−	−
NA09935A	−	−	+	+	+	+	−	−	−	(+)	−	+	+	+	−	−	+	+	−	(+)	+	+	−	−	−
NA09936	−	−	−	+	−	+	+	+	−	+	+	−	(+)	+	(+)	−	+	−	+	+	−	+	−	−	−
NA09937	−	−	+	+	−	+	+	+	−	(+)	−	+	−	+	+	−	+	+	−	−	−	−	−	−	−
NA09938	−	−	(+)	+	+	+	+	(+)	−	−	+	+	−	+	(+)	−	+	−	(+)	+	+	+	−	(+)	−
NA09940	−	−	+	−	−	−	+	+	−	−	−	−	−	−	+	−	+	−	−	−	−	−	−	−	−
NA10324	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−
NA10567	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−
NA10611	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Concordant[a]																									
+/+	4	3	3	4	2	4	4	4	0	2	0	2	3	4	4	1	4	4	4	4	1	2	0	1	
−/−	14	11	7	7	7	6	6	5	13	8	8	6	10	5	6	13	3	10	9	7	7	8	11	10	
Discordant[b]																									
+/−	0	1	1	0	1	0	0	0	4	1	3	1	0	0	0	2	0	0	0	0	2	1	3	3	
−/+	0	3	6	6	7	8	6	7	1	2	5	8	2	8	5	1	11	4	2	6	7	5	1	2	
% Discordant	0%	22%	41%	35%	47%	44%	38%	44%	28%	23%	50%	53%	13%	47%	33%	18%	61%	22%	13%	35%	53%	38%	27%	31%	

Note: (+) indicates presence of the human chromosome in less than 10% of metaphyses; these are excluded in concordance determination.
[a]Concordant hybrids have either retained (+/+) or lost (−/−) the human specific *COL9A2* band with a specific human chromosome.
[b]Discordant hybrids: Human specific *COL9A2* band present, the human chromosome absent (+/−), or the reverse (−/+).

100% concordance was seen for the cosegregation of the human-specific COL9A2 band and human chromosome 1 (Table I). All other autosomes and the sex chromosomes could be excluded by the presence of at least two discordant hybrids.

4. DISCUSSION

Comparison of the human α2(IX) collagen sequence (Fig. 2) with the corresponding chick sequence [21,22] revealed an amino acid identity of 78% and nucleotide identity of 71%. This corresponds well with the conservation between chick and human α1(IX) collagen sequences [11,21,22]. Interestingly, the amino acid sequence of the α2(IX) collagen exhibited marked conservation between chick and human in the NC3 domain near the serine residue which binds the glycosaminoglycan side chain [23]: only one amino acid substitution was seen in the entire NC3 domain of 17 amino acids. Also the location of the three discontinuities in the triple-helical sequence were conserved between chick and human although the one in COL3 domain is of the short type (Gly-X) in the mouse, and of the long type (Gly-X-Y-X-Y) in the chick (Fig. 2). Northern analyses of total RNAs from several human tissues confirmed that the 2.9 kb mRNA for α2(IX) collagen is only detected in cartilaginous tissues (data not shown).

The collagen gene family is known to be dispersed on several chromosomes [1]. Interestingly, also genes coding for the constituent chains of heterotrimeric collagen types (e.g. types I and XI) are located on different chromosomes although in some cases (types IV and VI) two coordinately expressed genes are found in the same locus. The gene for the human α1(IX) collagen has previously been mapped to chromosome 6 [11]. With the demonstration of the gene for the α2(IX) chain in chromosome 1 the genes for heterotrimeric type IX collagen must also be dispersed to at least two chromosomes. Molecular cloning of the α3(IX) collagen chain has only been performed for the chick [24,25]; therefore its chromosomal localization in the human remains unknown. Since each type IX collagen molecule is a heterotrimer of α1(IX), α2(IX) and α3(IX) chains the synthesis of these molecules presents interesting questions about their coordinated regulation. With the availability of new cDNA probes such questions can now be studied. The probes should be equally interesting for studies on the molecular genetics of human chondrodysplasias.

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