SHORT COMMUNICATION

Isao Nakamura · Akihiko Okawa · Shiro Ikegawa
Kunio Takaoka · Yusuke Nakamura

Genomic organization, mapping, and polymorphisms of the gene encoding human cartilage intermediate layer protein (CILP)

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Abstract The *CILP* gene encodes a proform of two polypeptides. One of them, cartilage intermediate layer protein (CILP), is a non-collagenous protein recently isolated from human articular cartilage. The other is homologous to a porcine nucleotide pyrophosphohydrolase (NTPPHase) whose enzymatic activity is highest in articular tissue. The investigation reported here revealed that the *CILP* gene consists of nine exons spanning approximately 15 kb of genomic DNA on chromosome 15q22. We also report six single nucleotide variations in this gene; five of them cause amino acid changes and the most common of them substitutes isoleucine for threonine at codon 395.

Key words Genomic organization · *CILP* · porcine *NTPPHase* · Polymorphism

Introduction

The human *CILP* gene encodes a 1 184-amino-acid proform of two polypeptides, cartilage intermediate layer protein (CILP) and a molecule homologous to porcine nucleotide pyrophosphohydrolase (NTPPHase; Lorenzo et al. 1998b).

CILP, a non-collagenous protein, was isolated recently from human articular cartilage. Its structure and function

have not been clarified; it is known, however, that CILP is synthesized by chondrocytes and is expressed emphatically in early stages of human osteoarthritis. CILP levels increase with age in articular cartilage. These findings suggest that CILP may play a role in maintaining tissue homeostasis in articular cartilage by controlling chondrocytes, and may also help to balance the processes of cartilage repair and degradation (Lorenzo et al. 1998a).

A porcine enzyme, chondrocyte nucleotide pyrophosphohydrolase (NTPPHase), exhibits its highest activity in articular tissue, i.e., in hyaline cartilage, ligaments, or tendons (Cardenal et al. 1996a). The human homologue of NTPPHase was identified by the same group (Cardenal et al. 1996b). Ecto-NTPPHase in joint tissues and synovial fluid produces inorganic pyrophosphate (PPi) from extracellular ATP (Rachow et al. 1988; Ryan et al. 1991; Park et al. 1996). The PPi concentration and the activity of NTPPHase are elevated in the synovial fluid of patients with calcium pyrophosphate dihydrate (CPPD) crystal-deposition disease (reviewed in Ryan and McCarty (1993); Masuda et al. 1995). To investigate a possible relationship between mutations or variants of the *CILP* gene and articular diseases, we mapped the gene and determined its genomic organization.

We synthesized a probe for NTPPHase cDNA from total RNA of porcine testis by means of reverse transcriptase-polymerase chain reaction (RT-PCR), labeled it with ³²P, and screened a cosmid library prepared from human peripheral lymphocytes. One isolated clone, designated N-55A, was fragmented by sonication, and 2.0- to 5.0-kb DNA fragments were subcloned into pBC. Two hundred randomly selected subclones were sequenced at both ends with T3 and T7 dye terminators (ABI Prism 377, Perkin-Elmer, Foster City, CA, USA), and DNA sequences were assembled by means of the ABI "Assembler" computer software. Through large-scale DNA sequencing in cosmid N-55A, we obtained 19 486 bp of genomic sequence (GenBank accession no. AB019040) and determined exon-intron boundaries by comparing the published cDNA sequence of the *CILP* (EMBL accession no. AF035408) with this genomic sequence (Table 1). The gene consisted of nine exons (Fig. 1a) and spanned approximately 15 kb of

I. Nakamura · A. Okawa · S. Ikegawa · Y. Nakamura (✉)
Laboratory of Molecular Medicine, Human Genome Center,
Institute of Medical Science, The University of Tokyo, 4-6-1
Shirokanedai, Minato-ku, Tokyo 108-8639, Japan.
Tel. +81-3-5449-5372; Fax +81-3-5449-5433
e-mail: yusuke@ims.u-tokyo.ac.jp

I. Nakamura · K. Takaoka
Department of Orthopaedic Surgery, Shinshu University School of
Medicine, Nagano, Japan

A. Okawa
Department of Orthopaedic Surgery, Chiba University School of
Medicine, Chiba, Japan

Table 1 Exon-Intron boundary sequence of the *CILP* gene

Exon number	Exon length (bp)	cDNA ^a position	Splice acceptor		Splice donor		Intron number	Intron length (bp)
1	23	1-23			TCAAGACACG	gtgagctcca	1	1566
2	167	24-190	ttctttacag	GTCAGTGGAT	TCTGTGTTGG	gtataggata	2	1932
3	93	191-283	tggccctcag	GGAGACAGAC	ACCCTGGAGA	gtgagttgtg	3	620
4	270	284-553	tgtctctcag	GCCCTGGTGA	TGCCCACCAG	gtaagccaga	4	1315
5	180	554-733	cactttgcag	GATCCCTGCG	GACTGTACAG	gtaccacct	5	704
6	315	734-1048	tgttccacag	CCTGTGACCT	GTGAGGGCAG	gtaactaat	6	797
7	109	1049-1157	tctgtccag	AGACTCATA	AGTATTTTGT	gtgcgtattc	7	1334
8	158	1158-1315	tccttgacag	GTATCATAAT	ATTGTCACAG	gtaagcctgt	8	2777
9	2848	1316-4163	ccctacacag	CATCTGATGA				

The GenBank accession number of this *CILP* nucleotide sequence is AB019040

^a the first nucleotide of the published cDNA sequence is designed as position 1

Fig. 1a, b **a** Structure of the *CILP* gene and its predicted protein product. *Top*: Genomic structure. Coding regions are denoted by filled boxes, and untranslated regions by open boxes. *Middle*: cDNA structure. *Bottom*: Schematic protein structure. CILP precursor protein is cleaved and yields cartilage intermediate layer protein (CILP) and nucleotide pyrophosphohydrolase (NTPPHase). **b** Chromosomal mapping of the *CILP* gene. Metaphase chromosomes stained with propidium iodide show twin-spot signals on the long arm of chromosome 15 (indicated by arrowheads)

Table 2 Sequence variation and allelic frequency of the *CILP* gene

Location	Nucleotide change	Amino acid ^a change	Allelic frequency(%)	Number of chromosomes examined
Intron 6	IVS6-12 (C→T)	intronic variant	254 (70.9) : 104 (29.1)	358
Exon 7	1109 (C→T)	S327F	356 (99.4) : 2 (0.6)	358
Exon 8	1313 (C→T)	T395 I	282 (78.8) : 76 (21.2)	358
Exon 9	2813 (C→T)	A895V	356 (99.4) : 2 (0.6)	358
Exon 9	3430 (C→T)	D1101N	937 (99.5) : 5 (0.5)	942
Exon 9	3632 (C→T)	V1168A	719 (99.9) : 1 (0.1)	720

^a The amino acid residue of the translation site is designed as position 1

genomic DNA. The sequences at all exon-intron boundaries conformed to the AG-GT consensus sequence for splicing boundaries. Exon 9, the longest exon, contained 2 848 base pairs. All of the partial sequence of porcine chondrocyte NTPPHase that was reported previously was present in exon 9 of the human homologue.

To determine the chromosomal location of this gene we performed fluorescence *in situ* hybridization (FISH) according to the procedure described by Inazawa et al. (1993). Cosmid N-55A was labeled with biotin-16-dUTP by nick-translation and hybridized to denatured human metaphase chromosomes; hybridization signals were rendered visible with FITC-avidin. Precise assignments of the signals were determined by visualization of the replicated G-bands. Signals were observed exclusively on band q22 of chromosome 15 (Fig. 1b).

We investigated the *CILP* gene for the presence of DNA polymorphisms in Japanese individuals and found six variants. Of these, five would substitute amino acids at codons 327, 395, 895, 1101, and 1168 respectively (Table 2). The frequencies of the minor alleles among at least 179 normal individuals were very low, except for the T395I substitution in exon 8.

Our structural analysis of the *CILP* gene may begin to shed some light on the etiology of joint diseases such as osteoarthritis or CPPD crystal-deposition disease.

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