J Hum Genet (2001) 46:456–462

ORIGINAL ARTICLE

**Akihiko Mabuchi · Toshiyuki Ikeda · Akira Fukuda
Yu Koshizuka · Hisatada Hiraoka · Kota Miyoshi
Nobuhiko Haga · Hiroshi Kawaguchi · Akira Kawakami
Seizo Yamamoto · Yoshio Takatori · Kozo Nakamura
Shiro Ikegawa**

Identification of sequence polymorphisms of the *COMP* (cartilage oligomeric matrix protein) gene and association study in osteoarthrosis of the knee and hip joints

Received: March 30, 2001 / Accepted: May 7, 2001

Abstract Osteoarthrosis (OA) is a common cause of musculoskeletal disability characterized by late-onset degeneration of articular cartilage. Although several candidate genes have been reported, susceptibility genes for OA remain to be determined. Hereditary osteochondral dysplasias produce severe, early-onset OA and hence are models for common idiopathic OA. Among them are pseudoachondroplasia and multiple epiphyseal dysplasia, both of which are caused by mutations in the cartilage oligomeric matrix protein (COMP) gene. Therefore, *COMP* may be a susceptibility gene for OA. We screened for polymorphisms by direct sequencing of all exons of the *COMP* gene with their flanking intron sequences and the promoter region. We identified 16 polymorphisms, of which 12 were novel. Using six polymorphisms spanning the entire *COMP* gene, we examined the association of *COMP* in Japanese patients with OA of the knee and hip joints. Genotype and allele frequencies of the polymorphisms were not significantly different between OA and control groups, and there was no significant difference in haplotypes. These results do not support an association between *COMP* and OA in the Japanese population.

Key words Polymorphism · SNP · COMP · Association study · Haplotype · Osteoarthrosis · Knee · Hip

A. Mabuchi · T. Ikeda · S. Ikegawa (✉)
Laboratory for Bone and Joint Diseases, SNP Research Center, RIKEN (The Institute of Physical and Chemical Research), c/o Institute of Medical Science, University of Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo 108-8639, Japan
Tel./Fax +81-3-5449-5393
e-mail: sikegawa@ims.u-tokyo.ac.jp

A. Fukuda · Y. Koshizuka · H. Hiraoka · H. Kawaguchi · Y. Takatori · K. Nakamura
Department of Orthopaedic Surgery, The University of Tokyo, Tokyo, Japan

K. Miyoshi
Department of Orthopedics, Yokohama Rosai Hospital, Yokohama, Japan

N. Haga
Department of Pediatric Orthopedics, Shizuoka Children's Hospital, Shizuoka, Japan

A. Kawakami
Department of Orthopedics, Tokyo Teishin Hospital, Tokyo, Japan

S. Yamamoto
Department of Orthopedic Surgery, Tokyo Metropolitan Geriatric Hospital, Tokyo, Japan

Introduction

Osteoarthrosis (OA) is a common cause of musculoskeletal disability related to aging and characterized by late-onset degeneration of articular cartilage. The prevalence of OA increases with age, and 10% of the elderly are affected with OA (Felson and Zhang 1998). A genetic contribution to OA has been suggested in several epidemiological studies (Stecher 1941; Kellgren et al. 1963; Spector et al. 1996; MacGregor et al. 2000). Genetic factors comprise the essential components of the etiology of OA; however, susceptibility genes for OA remain to be determined.

Many osteochondral dysplasias with Mendelian inheritance manifest involvement of articular cartilage and OA. These heritable diseases are models for OA as a common disease in a general population, and mutations in the causal gene of these diseases are clues to the susceptibility genes for OA. Among these osteochondral dysplasias that are associated with OA are pseudoachondroplasia (PSACH) and multiple epiphyseal dysplasia (MED). PSACH is characterized by severe disproportionate dwarfism, early-onset OA, and spinal deformity, whereas MED is characterized by early-onset OA and dysplasia of epiphyses with less-severe dwarfism. PSACH and MED are allelic, and are caused by mutations in the cartilage oligomeric matrix protein (COMP) gene (Briggs et al. 1995; Hecht et al. 1995).

Mutations in the *COMP* gene produce a phenotypic gradation, from severe PSACH to mild MED (Ikegawa et al. 1998). Late-onset milder MEDs are occasionally indistinguishable from common OA without information of posi-

tive family history and symmetric involvement of multiple joints (Spranger et al. 1974). Furthermore, a mutation in the C-terminus of COMP produces a normal height individual with skeletal abnormalities, including early OA (Briggs et al. 1998). These findings lead us to the hypothesis that OA as a common disease might be at the mild end of the phenotypic gradation produced by *COMP* mutations.

In the present study, we ascertained the sequences of the exons and exon–intron boundaries and identified 16 polymorphisms in the *COMP* gene. We investigated whether these polymorphisms were associated with OA of the knee (KOA) and hip (HOA) joints in Japanese.

Subjects and methods

Subjects

A total of 402 OA patients (195 KOA and 207 HOA) and 228 control individuals were included in this cross-sectional multi-center study. All individuals visited hospitals for treatment and agreed to participate in this study. Ethical approval was obtained from the ethical committee of each participating institution, and a written informed consent was obtained from all subjects.

KOA was diagnosed based on clinical symptoms and radiological examinations. Inclusion criteria for KOA were (1) age 50 years or more; (2) presence of symptoms, i.e. pain, claudication, and so on; (3) narrowing of the articular joint space; and (4) formation of osteophytes on one or both knees. HOA was diagnosed by clinical symptoms and radiological findings of narrowing of the joint space and/or osteophytes of the hip joint. Because a dysplastic hip is a major cause and predisposing factor of HOA in Japan (Nakamura et al. 1989), individuals with a dysplastic hip (center-edge angle $<25°$ or acetabular angle $>25°$ in the antero-posterior radiograph of the hip) were also included. All X-ray films were read by expert observers (A.F. for KOA and Y.T. for HOA). Most control individuals were patients with ossification of the posterior longitudinal ligament of the spine (OPLL). Genomic DNA of all individuals was extracted from 20 ml of peripheral blood using a Genomix kit (Talent, Trieste, Italy).

Determination of genomic structure and sequences of exon–intron boundaries

Genomic structure of *COMP* was determined by comparing published genomic and cDNA sequences (GenBank accession No. L32137) and was confirmed by direct sequencing of polymerase chain reaction (PCR) products from genomic DNA. PCR primers for exons 8–14 of *COMP* were as previously described (Ikegawa et al. 1998). Primers for the promoter region and for exons 1–7 and 14–19 are listed in Table 1.

PCR was performed with the Takara exTaq system (Takara Shuzo, Otsu, Japan) according to the instructions of the manufacturer in a total volume of 25 μl, using as templates 50–100 ng of each genomic DNA sample. PCR conditions were as follows: initial denaturation (94°C, 2 min) followed by 35 cycles of denaturation (94°C, 30 s), annealing (55°C–63°C according to the Tm of the primers, 30 s), extension (72°C, 30 s), and final extension (72°C, 5 min). PCR products were purified by Ultrafree-MC (Millipore, Bedford, MA, USA) and sequenced directly with an ABI 3700 automated sequencer and a Prism-Ready Reaction DyeDeoxy-Terminator Cycle-Sequencing Kit (ABI, Foster City, CA, USA).

Table 1. PCR primers used in this study for screening sequence polymorphisms of the *COMP* gene

Amplified region	Primer name	Sequence (5′ → 3 ′)	Product size (bp)
Promoter	COMP prm3/F	ACAAAGGCCCAGAATGACAC	1449
	COMP prm3/R	GCTGTGGGAACAGCCTGTAA	
Promoter	COMP prm2/F	TGGGCCTCAGTTTCTCTGTC	1156
	COMP prm2/R	GGTGTGGCTGTATCGCATC	
Promoter-exon 1	COMP prm/F	TGAAGCAAGAGGATGCGATA	558
	COMP prm/R	ACGTCCCCTACGAACAGTCC	
Exon 2, 3	COMP i1i3/F	CGAGACTAGAGGAGCGCAGT	583
	COMP i1i3/R	CTCTTTTCCTCCCCAGCTTT	
Exon 4	COMP i3i4/F	AAGCGGGACAGAGACTCAGA	489
	COMP i3i4/R	GAACACTCCCAGTGGAGGAA	
Exon 5–7	COMP i4i7/F	TCCACATCCGAGACACTACG	870
	COMP i4i7/R	GCACACTGTGGGAGAGTGTAAG	
Intron 9	COMP e9e10/R	GGCAGGAGGATGTGGACC	695
	COMP e9e10/R	CTTGTCCTCGTCCGTGTTG	
Exon 10–12	COMP i9i12/F	TTGCCTTCTCGACCTGATCT	1506
	COMP i9i12/R	GCGTTTTGTCAAAGGCTACC	
Exon 14–17	COMP e14i17/F	CAGGACGACTTTGATGCAGA	1733
	COMP e14i17/R	GGGACCAGGGTCACACAG	
Exon 18, 19	COMP i17e19/F	CTGGCCACTGAAGCTCTGA	463
	COMP i17e19/R	TCTGAGCCCTTCTCACTTCC	

Primers for exons 8–14 are as previously described (Ikegawa et al. 1998) and are omitted from this table
PCR, polymerase chain reaction; COMP, cartilage oligomeric matrix protein

Identification of polymorphisms

Sequence variations in the coding regions and flanking noncoding regions of the *COMP* gene and in the 2.7-kb promoter region were examined by direct sequencing of the PCR products from genomic DNA. More than 32 chromosomes from unrelated individuals were examined.

Genotyping

Single-nucleotide polymorphisms (SNPs) were genotyped with a fluorescence-based 5′-nuclease detection assay, or TaqMan assay (Holland et al. 1991; Livak et al. 1995), using as templates 10 ng of each genomic DNA sample in a total volume of 15 μl. PCR primers and probes for the TaqMan assay are listed in Table 2. Amplification conditions were as follows: initial activation (at 50°C, 2 min and then at 95°C, 10 min), followed by 40 cycles of denaturation (95°C, 15 s), annealing, and extension (62°C, 1 min). After PCR amplification, fluorescence was read by an ABI 7700 Sequence Detector.

A tetranucleotide repeat locus in intron 9 was amplified using a primer set of COMPe9i10/fl (5′-GTTCTTCTTT CGCCCCTCCTAGACC-3′) labeled with 6-FAM and COMPe9i10/n (5′-GGGTCCCCAATGAAAAGGTA-3′) and electrophoresed with the ABI 3700 automated sequencer. Alleles were sized and genotyped using a Genescan (version 3.5, ABI) and a Genotyper (version 3.7, ABI).

For SP15, the genotype was determined by PCR amplification and subsequent *Nae*I (Takara, Tokyo, Japan) restriction-endonuclease digestion of a 480-bp fragment. The PCR products were digested for 12 h with 2 U of *NaeI* per microgram of DNA, at 37°C, then electrophoresed on 4% NuSieve GTG agarose gels (BMA, Rockland, ME, USA).

Association study

Polymorphic loci for the association study were selected by the following criteria: (1) Allelic frequency, i.e., common polymorphisms were given priority; and (2) interval of the loci, i.e., physical distance between two neighboring polymorphic sites was within 6 kb. Allele frequencies were calculated for all polymorphic loci, and genotype frequencies for all SNPs. A χ-squared test was performed comparing each OA group and the control group. The odds ratio of the minor alleles was also calculated with a 95% confidential interval. Maximum-likelihood haplotype frequencies were calculated using an expectation-maximization algorithm (Excoffier and Slatkin 1995). Fisher's exact test of population differentiation was performed, comparing haplotype frequencies between each OA group and the control group (Rousset and Raymond 1995). Haplotypes were analyzed using Arlequin software (http://anthro.unige.ch/arlequin/).

Results

Nucleotide sequences of exon–intron boundaries

The entire exons and exon–intron boundaries of the *COMP* gene as well as the 2.7-kb promoter region were amplified from genomic DNA and sequenced directly. Sequences of the exon–intron boundaries predicted from the database were confirmed by sequencing the PCR products from genomic DNA, which all conformed to the AG–GT rule (Table 3).

Identification of polymorphisms

A total of 16 polymorphisms, 14 SNPs, one dinucleotide repeat, and one tetranucleotide repeat were detected

Table 2. Primers and probes used for the 5′-nuclease detection assay

SNP	Primer	Probe	Sequence (5′ → 3′)
−1417C/G	upst1417/F		TCCAGCTGCTGTGGGAACA
	upst1417/R		GTGAGTACTGGAAGGTGGGAGTG
		upst1417C	VIC-TGCGCGCCCGGCTTCT-TAMRA
		upst1417G	FAM-ACTGCGCGCCAGGCTTCTG-TAMRA
c.279C/A	ex4 c297/F		CCTACCCAGCGTGCGG
	ex4 c297/R		CGTCTGGATGCAGGCCA
		ex4 c297C	VIC-TGCGCGCCCGGCTTCT-TAMRA
		ex4 c297A	FAM-ACTGCGCGCCAGGCTTCTG-TAMRA
IVS5+76T/C	IVS5+76/F		CCCCATTTTTGGAGCAGAAG
	IVS5+76/R		CTGCCCCAAACCGATCAG
		IVS5+76T	VIC-AACTCCTCTTCCAGCCT-MGB
		IVS5+76C	FAM-CTCCTCTCCCAGCCT-MGB
IVS16−45C/T	IVS16−45/F		CTGCCAATACCCAGGAAAGG
	IVS16−45/R		TCCCAACTGTCTCTCCAGGAA
		IVS16−45C	VIC-TCGTGGGCCACCGGAGC-MGB
		IVS16−45T	FAM-CGTGGGCCACCAGAGCCC-MGB

SNP, single-nucleotide polymorphism

Table 3. Exon–intron boundary sequences of the *COMP* gene

Exon number	Exon size (bp)	cDNA position[a]	Splice acceptor site[b]	Splice donor site[b]	Intron size (bp)
1	104	−25–79		AGCCCGTTGGgtaagccgcg	255
2	86	335–420	gccgccgcagGCTCAGACCT	GCGGCAGCAGgtgcggggcc	236
3	52	657–708	acccctgcagGTCAGGGAGA	GACGCGTGCGgtgagcgcgg	447
4	173	1156–1328	ccccccgcagGGATGCAGCA	CGTCAACGAGgtgcgctagc	644
5	138	1973–2110	cctcccccagTGCAACGCCC	CAACAAGCAGgtgagaggtg	246
6	75	2357–2431	cctcccctagGTTTGCACGG	CAACACCCGGgtaaggcccg	88
7	159	2520–2678	tccggcgcagGGCTCCTTCC	GTCGTGCGTGgtgagtgcgg	77
8	105	2756–2860	tctcccacagTGTGCCGTTG	GTGCCGTAAGgtgggtgggg	90
9	108	2951–3058	ccgcccccagGACAACTGCG	CAATGAAAAGgtagatctac	561
10	160	3620–3779	tgtcatccagGACAACTGCC	GACGGCGACCgtgagctcga	839
11	119	4619–4737	atttctgcagGGATCCGCAA	CCCGGATCAGgtgagggcag	240
12	53	4978–5030	ccctccgcagGCGGATGTGG	ATCAAGACCAgtaaggaggc	92
13	182	5123–5304	tccactctagGGATGGAGAC	GACGCGGACAgtacggcggg	113
14	179	5418–5596	cacctctcagGGGACGGCGT	GCTCAACCAGgtgggagcgg	126
15	49	5723–5771	accccatcagGGAAGGGAGA	CTGGCTGTGGgtgagaagcg	405
16	197	6177–6373	ccatccctagGTTACACTGC	CCAACTCAAGgtgccagggc	532
17	173	6906–7078	gtattggcagGCTGTGAAGT	GCTACATCAGgtggggactc	997
18	140	8076–8215	tgaccctcagGGTGCGATTC	CGCTGCAATGgtgagccgag	92
19	188	8308–8495	ctccctgcagACACCATCCC		

[a] The translation start site of the *COMP* gene is designated as position 1
[b] Exon sequences are depicted by capital letters, and intron sequences by small letters

Table 4. List of polymorphisms of the *COMP* gene

No.	Location	Position[a]	Nucleotide change	Amino acid change	Frequency[b]	Reference
SP1	promoter	−2029	G/A		2/32	this study
SP2	promoter	−1730	A/T		5/32	dbSNP rs#81180
SP3	promoter	−1417	C/G		(0.18)	dbSNP rs#68760
SP4	promoter	−637	G/C		1/32	this study
SP5	promoter	−514	C/A		4/32	this study
SP6	promoter	−491	C/T		1/32	this study
SP7	promoter	−395	A/G		3/32	this study
SP8	promoter	−332	(CA)n repeat			this study
SP9	exon 4	c.279	C/A	Pro93Pro	(0.04)	Ikegawa et al. 1998
SP10	exon 5	c.468	G/T	Pro156Pro	1/32	this study
SP11	intron 5	IVS5+76	T/C		(0.05)	this study
SP12	intron 6	IVS6+39	T/C		1/32	this study
SP13	intron 9	intronic	(GAAA)n repeat			Briggs et al. 1995
SP14	intron 15	IVS15−185	G/C		1/32	this study
SP15	exon 16	c.1755	G/A	Thr585Thr	0/112[c]	Briggs et al. 1998
SP16	intron 16	IVS16−45	C/T		(0.07)	this study
SP17	intron 18	IVS18−40	T/C		(0.16)	this study

[a] The translation start site of the *COMP* gene is designated as position 1
[b] Figures in parentheses are overall frequency of the minor allele. Figures not in parentheses are the number of chromosomes carrying the minor allele per number of chomosomes sequenced
[c] SP15 was not detected in this study

(Table 4). Twelve of the polymorphisms were novel. A previously reported cSNP, c.1755G/A (Briggs et al. 1998) was not found in 112 chromosomes. Eight polymorphisms were identified in the promoter region of the *COMP* gene, two of which were within Alu repeats that extended 400 to 600 bp upstream from the translation start site. Three silent cSNPs were identified.

Association and haplotype analyses

The size of the *COMP* gene is about 8.5 kb. According to the selection criteria previously mentioned, five SNPs and one tetranucleotide repeat (−1417C/G in the promoter region, c.279C/A in exon 4, IVS5+76T/C, the tetranucleotide repeat in intron 9, IVS16−45C/T, and IVS18−40T/C) were used for the association study. The mean interval of the markers was 2.1 kb (range, 0.9–3.5 kb).

The distribution of the five SNPs in the KOA, HOA, and control groups is shown in Table 5. Observed genotype frequencies in these three groups were all in Hardy-Weinberg equilibrium. Allele and genotype frequencies between the OA and control groups did not differ significantly for any polymorphic loci. The locus with highest significance was SP9 in the HOA group ($\chi^2 = 3.79$, $P = 0.06$). Although polymorphisms in individuals with HOA carrying the minor allele of SP9 were screened, no

Table 5. Comparison of genotype and allele frequencies of SNPs of the *COMP* gene in osteoarthrosis of the knee (KOA) and hip (HOA) joints

SNP	Genotype/allele	KOA		HOA		Control
−1417G/G	Genotype 11	151 (0.68)		133 (0.69)		151 (0.64)
	12	64 (0.29)		53 (0.27)		79 (0.33)
	22	8 (0.04)	$P = 0.47$	8 (0.04)	$P = 0.29$	6 (0.03)
	Allele 1	366 (0.82)		319 (0.82)		381 (0.81)
	2	80 (0.18)	$P = 0.60$	69 (0.18)	$P = 0.57$	91 (0.19)
c.279C/A	Genotype 11	205 (0.94)		171 (0.90)		212 (0.94)
	12	12 (0.06)		19 (0.10)		13 (0.06)
	22	0 (0.00)	$P = 0.91$	1 (0.01)	$P = 0.15$	0 (0.00)
	Allele 1	422 (0.97)		361 (0.95)		437 (0.97)
	2	12 (0.03)	$P = 0.91$	21 (0.05)	$P = 0.06$	13 (0.03)
IVS5+76T/C	Genotype 11	202 (0.89)		177 (0.91)		215 (0.90)
	12	26 (0.11)		17 (0.09)		25 (0.10)
	22	0 (0.00)	$P = 0.73$	1 (0.01)	$P = 0.46$	0 (0.00)
	Allele 1	430 (0.94)		371 (0.95)		455 (0.95)
	2	26 (0.06)	$P = 0.74$	19 (0.05)	$P = 0.82$	25 (0.05)
IVS16−45C/T	Genotype 11	194 (0.85)		166 (0.85)		216 (0.89)
	12	32 (0.14)		28 (0.14)		26 (0.11)
	22	2 (0.01)	$P = 0.44$	2 (0.01)	$P = 0.38$	1 (0.00)
	Allele 1	420 (0.92)		360 (0.92)		458 (0.94)
	2	36 (0.08)	$P = 0.19$	32 (0.08)	$P = 0.16$	28 (0.06)
IVS18−40T/C	Genotype 11	160 (0.70)		140 (0.72)		159 (0.70)
	12	60 (0.26)		47 (0.24)		63 (0.28)
	22	8 (0.04)	$P = 0.83$	7 (0.04)	$P = 0.64$	6 (0.03)
	Allele 1	380 (0.83)		327 (0.84)		381 (0.84)
	2	76 (0.17)	$P = 0.93$	61 (0.16)	$P = 0.78$	75 (0.16)

Genotypes "11" and "22" are homozygotes of major and minor alleles, respectively
Genotype "12" is a heterozygote of major and minor alleles
Alleles 1 and 2 are major and minor alleles, respectively

polymorphisms other than those in the first screen were found.

A total of 19 alleles were identified in the tetranucleotide repeat polymorphism. Because 13 of the alleles were individually very rare, they were pooled and subjected to the analysis. Allele frequencies in the KOA group did not differ significantly from the control group ($\chi^2 = 0.80$, $P = 0.67$), nor did they in the HOA group ($\chi^2 = 1.03$, $P = 0.60$).

Haplotype analysis revealed that one major haplotype carrying each major allele was dominant in each group. Haplotype frequencies of the KOA and HOA groups were not different from those of the control subjects (Fisher's exact test P was 0.98 for the KOA group and 0.57 for the HOA group).

Discussion

We hypothesize that *COMP* is a candidate gene for OA. Histology of OA reveals qualitative and quantitative abnormalities of the extracellular matrix of the articular cartilage, which is mainly composed of collagens and non-collagenous proteins. COMP is a major component of the latter (Hedbom et al. 1992) and is thought to play a major role in development and homeostasis of the cartilage (DiCesare et al. 1994). The association of OA with *COL2A1*, which encodes type II collagen, the major collagen of the articular cartilage, has been reported (Knowlton et al. 1990). Hence, it is reasonable to speculate that abnormal COMP, another major component of the cartilage matrix, could also cause OA. Actually, the *COMP* mutation produces early-onset OA in PSACH and MED.

In this study, we identified 16 polymorphisms, including 12 novel ones, in the *COMP* gene. We set the maximal interval between the marker loci to less than 6 kb, because a simulation study suggests that the useful range of linkage disequilibrium (LD) in the general population is as short as 3 kb (Kruglyak 1999), which is to our knowledge the shortest range proposed. Estimation of LD based on real data has usually given broader ranges (Clark et al. 1998; Abecasis et al. 2001). Using this dense set of polymorphisms, we investigated the association between the *COMP* genotypes and the prevalence of OA of the knee and hip joint in the Japanese population. However, we could not find evidence for a positive association between the *COMP* gene and the two types of OA.

Our study has several limitations. The first is the range of the genomic region screened for polymorphisms. We detected polymorphisms in the entire exons and in the exon–intron boundary portions of the introns, but not elsewhere in the introns. Intronic polymorphisms that escaped detection may affect the prevalence of OA, although haplotype analysis results were negative for that hypothesis.

The second is the number of chromosomes screened for polymorphisms. We used at least 32 chromosomes for detection. That number guaranteed identification of sequence variations with frequencies of >10% (relatively common

polymorphisms) at more than 95% probability. We aimed to isolate relatively common polymorphisms because uncommon polymorphisms require a huge number of samples for detection of association and are in practice uninformative (Risch and Merikangas 1996). In our attempt to detect PSACH and MED mutations using genomic DNA and cDNA, we found no other exonic polymorphisms, even though the entire coding region of *COMP* was examined in 19 patients (Mabuchi et al. to be published).

The third limitation is that loci with significant association may exist in a region more proximal or distal to the region we screened, i.e., in the 5′ or 3′ flanking regions. A recent study of chondrocyte-specific expression of the murine COMP gene reveals that two regulatory elements in the 5′ flanking region and the proximal element are located as far as 1.9 kb upstream from the transcription start site (Issack et al. 2000). However, our search covered the corresponding region of the human *COMP* gene. Polymorphisms we identified in the promoter region were in considerable LD and were well represented by the −1417C/G polymorphism.

It is of note that the estimated haplotypes in our study include one major and several minor ones. If the major haplotype carries a significant polymorphism with low frequency, it might mask contributions by other significant polymorphisms in the case-control study. However, polymorphisms other than those examined in our study should not exist, or even if they do exist, their frequency would be very low.

The absence of positive association in the present study is consistent with previous linkage studies. Mustafa et al. [2000] reported a P value of <0.05 in a stratified linkage analysis using D19S215, but did not confirm the relationship by multiple testing. There is no suggestion of a linkage in a sibling-pair study of chromosome 19, which carries the *COMP* gene [Loughlin et al. 1999]. Taken together with our result, *COMP* is probably not a major cause of OA. The limited sample size of our study might preclude detection of a small contribution of *COMP* polymorphism(s) to a small number of OA patients. This possibility will be clarified by future large-scale association studies.

Acknowledgments We thank the patients, who cooperated in this study. We also thank Drs. Hideto Machida, Yuji Okazaki, and Yoji Mikami for collecting samples, Ms. Yoshie Takanashi for excellent technical assistance, and Mrs. Hiromi Kakoi for preparing the manuscript.

References

Abecasis GR, Noguchi E, Heinzmann A, Traherne JA, Bhattacharyya S, Leaves NI, Anderson GG, Zhang Y, Lench NJ, Carey A, Cardon LR, Moffatt MF, Cookson WO (2001) Extent and distribution of linkage disequilibrium in three genomic regions. Am J Hum Genet 68:191–197

Briggs MD, Hoffman SMG, King L, Olsen AS, Mohrenweiser H, Leroy JG, Mortier GR, Rimoin DL, Lachman RS, Gaines ES, Cekleniak JA, Knowlton RG, Cohn DH (1995) Pseudoachondroplasia and multiple epiphyseal dysplasia due to mutations in the cartilage oligomeric matrix protein gene. Nat Genet 10:330–336

Briggs MD, Mortier GR, Cole WG, King LM, Golik SS, Bonaventure J, Nuytinck L, De Paepe A, Leroy JG, Biesecker L, Lipson M, Wilcox WR, Lachman RS, Rimoin DL, Knowlton RG, Cohn DH (1998) Diverse mutations in the gene for cartilage oligomeric matrix protein in the pseudoachondroplasia-multiple epiphyseal dysplasia disease spectrum. Am J Hum Genet 62:311–319

Chitnavis J, Sinsheimer JS, Clipsham K, Loughlin J, Sykes B, Burge PD, Carr AJ (1997) Genetic influences in end-stage osteoarthritis. Sibling risks of hip and knee replacement for idiopathic osteoarthritis. J Bone Joint Surg Br 79:660–664

Clark AG, Weiss KM, Nickerson DA, Taylor SL, Buchanan A, Stengard J, Salomaa V, Vartiainen E, Perola M, Boerwinkle E, Sing CF (1998) Haplotype structure and population genetic inferences from nucleotide-sequence variation in human lipoprotein lipase. Am J Hum Genet 63:595–612

DiCesare P, Hauser N, Lehman D, Pasumarti S, Paulsson M (1994) Cartilage oligomeric matrix protein (COMP) is an abundant component of tendon. FEBS Lett 7;354:237–240

Excoffier L, Slatkin M (1995) Maximum-likelihood estimation of molecular haplotype frequencies in a diploid population. Mol Biol Evol 12:921–927

Felson DT, Zhang Y (1998) An update on the epidemiology of knee and hip osteoarthritis with a view to prevention. Arthritis Rheum 41:1343–1355

Hecht JT, Nelson LD, Crowder E, Wang Y, Elder FFB, Harrison WR, Francomano CA, Prange CK, Lennon GG, Deere M, Lawler J (1995) Mutations in exon 17B of cartilage oligomeric matrix protein (COMP) cause pseudoachondroplasia. Nat Genet 10:325–329

Hedbom E, Antonsson P, Hjerpe A, Aeschlimann D, Paulsson M, Rosa-Pimentel E, Sommarin Y, Wendel M, Oldberg A, Heinegard D (1992) Cartilage matrix proteins. An acidic oligomeric protein (COMP) detected only in cartilage. J Biol Chem 25;267:6132–6136

Holland PM, Abramson RD, Watson R, Gelfand DH (1991) Detection of specific polymerase chain reaction product by utilizing the 5′–3′ exonuclease activity of Thermus aquaticus DNA polymerase. Proc Natl Acad Sci U S A 15;88:7276–7280

Ikegawa S, Ohashi H, Nishimura G, Kim KC, Sannohe A, Kimizuka M, Fukushima Y, Nagai T, Nakamura Y (1998) Novel and recurrent COMP (cartilage oligomeric matrix protein) mutations in pseudoachondroplasia and multiple epiphyseal dysplasia. Hum Genet 103:633–638

Issack PS, Fang C, Leslie MP, Di Cesare PE (2000) Chondrocyte-specific enhancer regions in the COMP gene. J Orthop Res 18:345–350

Kellgren JH, Lawrence JS, Bier F (1963) Genetic factors in generalised osteoarthritis. Ann Rheum Dis 22:237–255

Knowlton RG, Katzenstein PL, Moskowitz RW, Weaver EJ, Malemud CJ, Pathria MN, Jimenez SA, Prockop DJ (1990) Genetic linkage of a polymorphism in the type II procollagen gene (COL2A1) to primary osteoarthritis associated with mild chondrodysplasia. N Engl J Med 22;322:526–530

Kruglyak L (1999) Prospects for whole-genome linkage disequilibrium mapping of common disease genes. Nat Genet 22:139–144

Livak KJ, Flood SJ, Marmaro J, Giusti W, Deetz K (1995) Oligonucleotides with fluorescent dyes at opposite ends provide a quenched probe system useful for detecting PCR product and nucleic acid hybridization. PCR Methods 4:357–362

Loughlin J, Mustafa Z, Irven C, Smith A, Carr AJ, Sykes B, Chapman K (1999) Stratification analysis of an osteoarthritis genome screen — suggestive linkage to chromosomes 4, 6, and 16. Am J Hum Genet 65:1795–1798

MacGregor AJ, Antoniades L, Matson M, Andrew T, Spector TD (2000) The genetic contribution to radiographic hip osteoarthritis in women: results of a classic twin study. Arthritis Rheum 43:2410–2416

Mustafa Z, Chapman K, Irven C, Carr AJ, Clipsham K, Chitnavis J, Sinsheimer JS, Bloomfield VA, McCartney M, Cox O, Sykes B, Loughlin J (2000) Linkage analysis of candidate genes as susceptibility loci for osteoarthritis — suggestive linkage of COL9A1 to female hip osteoarthritis. Rheumatology (Oxford) 39:299–306

Nakamura S, Ninomiya S, Nakamura T (1989) Primary osteoarthritis of the hip joint in Japan. Clin Orthop 241:190–196

Risch N, Merikangas K (1996) The future of genetic studies of complex human diseases. Science 273:1516–1517

Rousset F, Raymond M (1995) Testing heterozygote excess and deficiency. Genetics 140:1413–1419
Spector TD, Cicuttini F, Baker J, Loughlin J, Hart D (1996) Genetic influences on osteoarthritis in women: a twin study. BMJ 312:940–943
Spranger J, Langer LO, Wiedemann J-R (1974) Bone dysplasias. Gustav Fisher Stuttgart
Stecher RM (1941) Heberden's nodes: heredity in hepertrophic arthritis of finger joints. Am J Med Sci 201:801–809