Association of Chondromodulin-II Val58Ile Polymorphism with Radiographic Joint Destruction in Rheumatoid Arthritis

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ABSTRACT. ***Objective.*** Chondromodulin-II (ChM-II) is a cartilage-derived protein involved in cartilage and bone repair. A study of Japanese patients with rheumatoid arthritis (RA) implicated an association between a 172G → A (Val58Ile) polymorphism and radiographic damage. We analyzed ChM-II for polymorphisms and investigated the association with radiographically assessed joint destruction in German patients with RA. Possible interactions with the shared epitope (SE) were examined.

Methods. DNA samples from 204 patients with RA, 81 patients with osteoarthritis, and 116 patients with gout, serving as controls, were sequenced. Radiographic damage was assessed by modified Larsen score. Allele and genotype frequencies between groups were compared by Cochrane-Armitage trend tests.

Results. Five missense mutations, one silent mutation, and 5 intronic polymorphisms were found. Allele and genotype frequencies were similar in both disease groups. Larsen scores were significantly higher in RA patients carrying the 172AA (Ile/Ile) genotype (Larsen 96.8), than in RA patients with the 172GA (Val/Ile; Larsen 69.5) or 172GG (Val/Val; Larsen 54.8; p = 0.001) genotypes. Odds ratios to develop more severe radiographic joint damage (Larsen score > 90; above 75th percentile) were 4 and 15.5 for the 172GA and 172AA genotypes, respectively. Presence of a 172A allele increased the risk for enhanced radiographic damage 3-fold. SE and ChM-II 172A alleles emerged as 2 independent risk factors. A potentiated interaction of these risk alleles could not be verified.

Conclusion. Our data indicate that ChM-II Val58Ile polymorphism is associated with radiographic progression of joint destruction, particularly in German patients with RA negative for SE. (J Rheumatol 2005;32:1654–61)

Key Indexing Terms:
RHEUMATOID ARTHRITIS GENETICS CANDIDATE GENE
CHONDROMODULIN-II LEUKOCYTE CELL-DERIVED CHEMOTAXIN 2

Cartilage and bone destruction are key pathophysiological processes determining progression and outcome of rheumatoid arthritis (RA). Tumor necrosis factor-α (TNF-α) and interleukin 1 (IL-1) have been found to be mediators of cartilage destruction[1] and osteoclastic bone resorption[1-3]. Growth factors like transforming growth factor-ß (TGF-ß) and bone morphogenetic proteins (BMP), originating from the cartilage and the surrounding synovial tissue, may have a major role in repair reactions after cartilage and bone damage[4]. Recently, several cartilage-derived growth-promoting proteins have been isolated from the cartilage matrix[5].

Chondromodulin-II, a 16 kDa protein, is quite abundant in fetal cartilage. It enhances chondrocyte and osteoblast proliferation and stimulates proteoglycan synthesis[6,7]. It also promotes osteoclast differentiation[8]. Chondromodulin-II is identical to leukocyte cell-derived chemotaxin 2 (LECT2), which was isolated from liver and has been shown to be chemotactic for neutrophils *in vitro*[9]. The human gene is found on chromosome 5q31.1-32[10]. It spans roughly 8 kb and consists of 4 exons, encoding for 151 amino acids, and 3 introns[10].

Recently, an association between a G/A polymorphism at nucleotide position 172 (exon 3) in chondromodulin-II/LECT2 (ChM-II) gene, encoding a valine/isoleucine (Val/Ile) substitution, and RA has been reported for a Japanese population[11]. The A (Ile)-allele frequency was found to be significantly more prevalent in RA patients than controls. Further, the A (Ile)-allele was associated with more severe radiographic damage in Japanese patients with RA[11].

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We intended to validate this association in a clinically well characterized population of German Caucasians and to analyze the relation with their HLA (DRB1) status.

MATERIALS AND METHODS

Patients. The association between ChM-II polymorphisms and RA severity was studied in a group of 127 unrelated Caucasian patients with RA, all of German origin and resident in the Dresden territory, Saxony. The source group for all patients consisted of 204 patients who had been recruited at the outpatient department of the Department of Rheumatology (Carl Gustav Carus Medical School, University of Technology Dresden). Each participant gave written informed consent and the study was approved by the Dresden Ethics Committee according to the Declaration of Helsinki principles. From the initial sampling 9 patients were excluded with disease duration < 3 years and 68 patients with incomplete radiographs of hands, wrists, and forefeet, or missing HLA genotyping.

Demographic data are displayed in Table 1. All patients had been diagnosed with RA as classified by the 1987 American College of Rheumatology criteria[12] by a rheumatologist and were receiving antiinflammatory and/or antirheumatic chemotherapy. Most patients (99%) had been treated with one or more disease modifying antirheumatic drugs (DMARD). Eighty percent of patients were being treated with prednisolone. The current treatment rates for DMARD and prednisolone were 97% (mean 1.5 DMARD) and 69%, respectively.

The German control group consisted of 81 Caucasian patients with osteoarthritis (OA) and 116 patients with gout from the same outpatient department. All patients gave written consent.

HLA-DRB1 typing. HLA-DRB1 typing was performed by polymerase chain reaction (PCR) using a panel of sequence-specific oligonucleotide probes. HLA-DRB1*04 subtyping was performed with PCR-single-strand conformational probes. Patients were classified as shared epitope (SE)-positive if they carried DRB1*0101, *0102, *0401, *0404, *0405, *0408, or *1001. Genotyping of the HLA-DRB1 locus revealed 66 RA patients carrying one and 23 RA patients carrying 2 SE alleles.

Radiographic scoring. Radiographic outcome, recorded at final review, was obtained by scoring radiographs of the patients' hands and feet, using anteroposterior and laterolateral radiographs, with the method of Larsen modified by Rau and Herborn[13]. Thirty-two joints were evaluated: wrist, metacarpophalangeal, proximal interphalangeal, and first interphalangeal joints of the hands and the metatarsophalangeal joints of the feet. A damage score varying from 0 to 5 was attributed semiquantitatively to each joint, summing to a total score of 0 to 160. While 0 represents the normal joint, Grade 1 is characterized by soft tissue swelling and additional subchondral osteoporosis, Grade 2 represents destruction (erosion) of the joint surface of up to 25%, Grades 2 to 5 represent destruction of 26% to 50%, 51%–75%, and > 75%, respectively.

Table 1. Demographic profile and basic clinical and laboratory measures of the study population. Presence of HLA-DRB1 shared epitope was defined for DRB1*0101, *0102, *0401, *0404, *0405, *0408, or *1001.

Characteristic	N = 127
Male/female, n (%)	24 (18.9)/103 (81.1)
Age, median, yrs (range)	63 (27–87)
Age at onset, median, yrs (range)	48 (19–85)
Disease duration, median, yrs (range)	13 (3–47)
Rheumatoid factor, ever positive, %	91.3
Nodule-positive, %	26.0
Antinuclear antibodies-positive, %	37.8
Shared epitope status, n (%)	
–/–	38 (29.9)
–/+	66 (52.0)
+/+	23 (18.1)

Genomic DNA isolation. Total genomic DNA was extracted from white blood cells using a genomic DNA isolation kit (NucleoSpin Blood DNA extraction kit, Macherey-Nagel, Dueren, Germany) and proteinase K (Roche, Penzberg, Germany) at a final concentration of 1 μg/μl for 10 min at 70°C. The concentration of genomic DNA was determined by measuring the A_{260nm} in an MBA 2000 (Perkin Elmer, Foster City, CA, USA).

PCR amplification. Primer sequences used for amplification, annealing temperatures, and Mg^{2+} concentrations are shown in Table 2. The standard reaction mixture for exons 1–3 contained 10× PCR buffer (Perkin Elmer), 0.150 mM dNTPs (Amersham Pharmacia Biotech, Piscataway, NJ, USA), 1.0 μM primers, 0.5 U Taq (Perkin Elmer), and 50 ng genomic DNA final concentration, in a total volume of 25 μl. PCR was performed in a GeneAmp PCR System 9600 (Perkin Elmer) or in a T-Gradient Cycler (Biometra, Göttingen, Germany) with a cycle sequence of 94°C for 5 min, 95°C for 20 s, 35 cycles of 95°C for 30 s, 61.0°C for 40 s, extension at 72°C for 45 s, and 72°C for 10 min. Amplification of exon 4 was carried out with the Boehringer Mannheim Expand Long Template PCR Kit in system 2 as suggested by the supplier under the following conditions: 94°C for 2 min; 35 cycles of 94°C for 15 s, 60°C for 45 s, 68°C for 45 s; followed by 68°C for 10 min. Subsequently, the products were analyzed on 1.6% ethidium bromide agarose gel with a ClonTech 100 base pair ladder as molecular weight standard.

Sequencing. All exons were sequenced using a Thermo Sequenase fluorescent labeled primer cycle sequencing kit with 7-deaza-dGTP (USB, product number 78500) on ALF Express (Amersham Biosciences). Following the manufacturer's directions the sequence reaction was performed with 1 pmol of Cy5-labeled primer (same sequences as PCR primers shown in Table 2) and roughly 100 fmol of purified PCR product. The following cycle sequence was used: 94°C for 5 min, 25 cycles of 95°C for 20 s, 59°C for 40 s (exon 4: 57°C), and 72°C for 45 s. Polymorphisms were identified by sequence alignment with the sequence of human gene for LECT2 (NCBI Genbank accession number AB007546).

Statistical evaluations. To evaluate the deviation from Hardy-Weinberg equilibrium, observed and expected genotype frequencies were compared by an exact goodness-of-fit test in the total study population as well as separately in cases and controls. Allele and genotype frequencies between groups were compared by Cochrane-Armitage trend tests. Odds ratios, exact 95% confidence intervals, and exact p values are presented.

Frequencies of haplotypes constructed from 71T → C, 172G → A, and –36T → A polymorphisms were estimated with the expectation-maximization algorithm[14].

Associations between the ChM-II Val58Ile polymorphism and severity of disease by modified Larsen score were assessed using univariate analyses of variance (ANOVA) with subsequent DUNCAN multiple-range tests. Larsen scores were corrected for sex, age at disease onset, and disease duration (SPSS 11.0 for Windows; SPSS Inc., Chicago, IL, USA). A value of $p \leq 0.05$ was considered statistically significant.

RESULTS

Genotyping. Genotyping the ChM-II gene of 401 individuals (204 with RA, 81 with OA, 116 with gout) revealed 11 single-nucleotide polymorphisms (SNP; Table 3). One silent mutation (255T → C) and 5 missense mutations (71T → C, 172G → A, 188G → A, 205C → G, 247A → G) coding for amino acid substitutions Ile24Thr, Val58Ile, Gly63Glu, Pro69Ala, and Asn83Asp were found in exons 2 and 3 (Table 3). Both the allele and genotype frequencies of the detected mutations and polymorphisms were similar in the 2 study groups. Additionally, 5 nucleotide substitutions were

Table 2. Primer sequences (5'–3') and PCR conditions used for amplification of coding regions of chondromodulin-II gene. PCR was performed by Boehringer Mannheim Expand Long Template PCR Kit in system 2.

Exon	Size of PCR Product, bp	Primer Sequences (F: forward; R: reverse)	Annealing Temperature, °C	Mg^{2+}, mM
1	278	F AATGAGAGATGCAGAATTCTAAGGCTAA R AAGTCTTTGTTGTGATCATGCTATTAA	61.0	1.5
2	249	F AACTGTGTGACTGATAAGGCAA R AACGTTGGGCAAGGACACTAA	61.0	1.5
3	304	F AACACCATGTTCCCAGGCAGTAA R AATCTCTACGTATGGAACCTGCATTAA	61.0	1.5
4	372	F ATTCCTCTCCTGAGTTCATTCAACA R AACACAAATTTCTTGAAGAGAAGGGTA	60.0	2.25

Table 3. Polymorphisms in chondromodulin-II gene in patients with rheumatoid arthritis (RA), osteoarthritis (OA), and gout.

	Nucleotide	Protein	Frequencies of the Mutated Alleles, % RA, n = 204	OA, n = 81	Gout, n = 116	Significance**
Exon 2	71T>C	Ile24Thr	5.1	4.9	4.3	NS
Exon 3	172 G>A	Val58Ile	31.2	33.7	37.5	NS
	188 G>A	Gly63Glu	2.4	3.1	3.0	NS
	205 C>G	Pro69Ala	0.2	ND	ND	
	247 A>G	Asn83Asp	0.2	1.2	0.4	NS
	255 T>C	Asn85Asn	1.0	1.8	0.4	NS
Intron 1	+8 G>A		0.2	ND	ND	
Intron 2	–33 T>A		0.2	ND	ND	
	–19 G>A		0.5	1.2	ND	NS
Intron 3	–36 T*>A		72.0	73.4	76.7	NS
	–17 T>C		0.2	ND	ND	

* In Genbank sequence the T allele was stated as the wt-allele (Accession No. AB007546). ** Chi-square test. ND: not detected, NS: not significant.

detected in flanking intron regions (Table 3), with no differences in allele frequencies between the RA, OA, and gout groups.

The distribution of the 58Ile alleles in our German population (RA: 31.2%, OA: 33.7%, gout: 37.4%) was comparable to that of healthy Japanese individuals (34.7%)[11]. Although there was a tendency to a higher 58Ile allele frequency in the Japanese RA group (41.0%), this difference was not statistically significant in a metaanalysis by chi-square test including all German and Japanese data (Pearson: 11.277). Indeed, we could not confirm the Japanese finding of higher frequency of 58Ile in patients with RA, at least for the German population we studied.

Effects of SE and clinical characteristics on degree of radiographic joint destruction. RA patients carrying 2 HLA-DRB1 risk alleles had a significantly lower age of disease onset (characterized by age at diagnosis) in comparison to RA patients with one SE allele or none: 40.1 years (95% CI 34.3–45.9) compared to 49.3 years (95% CI 45.7–53.0) and 46.8 years (95% CI 42.8–50.9) ($p < 0.05$). The degree of radiographic joint destruction, assessed by modified Larsen score (data adjusted for sex, disease duration, and age at onset), was significantly higher in RA patients with 2 SE alleles [88.9 (95% CI 74.4–103.4)] than in RA patients with one SE allele [61.9 (95% CI 53.5–70.3)] or no SE allele [57.8 (95% CI 46.7–68.8)] ($p < 0.01$), respectively.

RA patients with rheumatoid nodules displayed a significantly higher Larsen score than RA patients without nodules: 59.1 (95% CI 52.2–66.2) compared to 83.8 (95% CI 71.9–95.6) ($p < 0.001$), respectively. There was no statistically significant difference in radiographic joint destruction between RA patients with and those without rheumatoid factor and antinuclear antibodies.

Association of ChM-II Val58Ile genotype and radiographic joint destruction. Except for the Val58Ile polymorphism, there were no significant differences in Larsen scores between ChM-II genotypes of RA patients. Larsen scores, adjusted for sex, age at disease onset, and disease duration, were significantly higher in RA patients carrying the Ile/Ile genotype [96.8 (95% CI 75.2–118.5)] than in RA patients with the Val/Ile [69.5 (95% CI 61.0–78.1)] or Val/Val [54.8 (95% CI 45.5–64.2)] ($p = 0.001$) genotypes (Figure 1). Estimating differences of allele and genotype frequencies by Cochrane-Armitage trend test in terms of enhanced joint destruction (Larsen score ≥ 90; i.e., 75th percentile) revealed a roughly 3-fold increase of the odds ratio for the

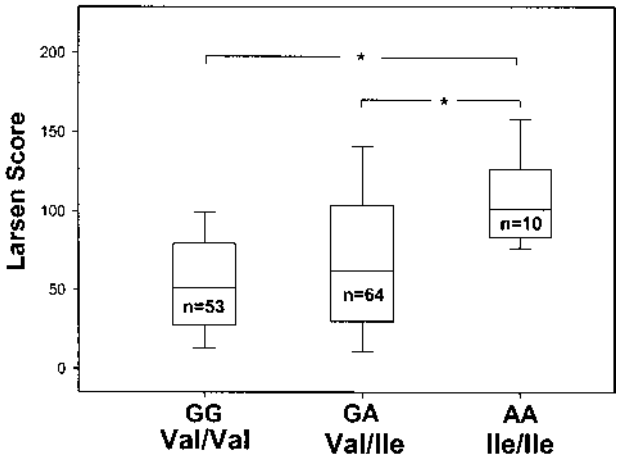

Figure 1. Larsen scores, adjusted for sex, age at disease onset, and disease duration, of the 3 chondromodulin-II Val58Ile genotypes in patients with RA. Box plots show the 10th, 25th, 50th, 75th, and 90th percentiles. *p ≤ 0.05.

172 A-allele (Table 4). The odds ratios to develop more severe radiographic joint damages were 4 and 15.5 for the GA and AA genotypes of the Val58Ile polymorphism, respectively (Table 4).

To investigate the effect of the 58Ile allele on the time course of radiographic joint destruction, RA patients were stratified into quartiles of disease duration: first quartile 3–7 years, second quartile 8–14 years, third quartile 15–23 years, and fourth quartile > 23 years. There was a tendency to higher Larsen scores for the heterozygote and homozygote 58Ile allele carrier in all subgroups, reaching statistical significance in quartiles 2 and 4, due to the number of observations per genotype.

Of particular interest was the analysis of an interaction between HLA status and ChM-II Val58Ile genotypes in terms of radiographic joint destruction. In RA patients negative for the shared epitope, the ChM-II 58Ile/Val and 58Ile/Ile genotypes were characterized by significantly higher Larsen scores than the 58Val/Val genotype (ANOVA, p = 0.003; Figure 2). The presence of one or 2 SE alleles resulted in a significant increase in Larsen score for RA patients with a ChM-II Val/Val genotype (ANOVA, p = 0.001; Figure 2). As a result of higher Larsen scores in RA patients with the ChM-II Val/Val genotype and one or 2 SE alleles, no potentiating effect could be observed for the ChM-II Val/Ile and Ile/Ile genotypes. Vice versa, the increase of Larsen scores for RA patients with the ChM-II Val/Ile and Ile/Ile genotypes blunted the potentiating effect of the SE.

In a model of univariate ANOVA with HLA status and ChM-II genotype as factors, and sex, age at disease onset, and disease duration as covariates, ChM-II (p = 0.002) and HLA (p = 0.042) exerted significant and independent effects on Larsen score. Further, the interaction between HLA status and ChM-II genotype had no significant effect in this model.

Construction of ChM-II haplotypes and their association with radiographic joint destruction. Applying genotypes of the 3 most frequent polymorphisms (71T → C, 172G → A, –36T → A; Table 3), 6 haplotypes were deduced, which are summarized in Table 5. There was no deviation from the Hardy-Weinberg equilibrium for any of the SNP (71T → C: chi-square = 0.618, p = 0.432; 172G → A: chi-square = 1.597, p = 0.206; –36T → A: chi-square = 0.994, p = 0.319) and all were in strong linkage disequilibrium. No significant differences were found between haplotype frequencies in RA, OA, and gout patients. Interestingly, the homozygote

Table 4. Analysis of allele and genotype frequencies in RA patients with Larsen score < 90 (controls) and ≥ 90 (cases) by Cochrane-Armitage trend test.

Exon/Intron	SNP	Allele/Genotype	Controls, n	Controls, %	Cases, n	Cases, %	OR	95% CI	p
Exon 2	71 T>C	T	183	96.3	62	96.9	1.000		
		C	7	3.7	2	3.1	0.843	0.083, 4.590	1.0000
		TT	88	92.6	30	93.8	1.000		
		TC	7	7.4	2	6.2	0.839	0.081, 4.741	1.0000
		CC	0	0.0	0	0.0			
Exon 3	172 G>A	G	139	73.2	31	48.4	1.000		
		A	51	26.8	33	51.6	2.901	1.545, 5.430	**0.0006**
		GG	47	49.5	6	18.8	1.000		
		GA	45	47.4	19	59.4	3.936	1.818, 9.415	
		AA	3	3.1	7	21.8	15.500	3.307, 88.64	**< 0.004**
Intron 3	–36 T>A	T	128	69.6	49	79.0	1.000		
		A	56	30.4	13	21.0	0.606	0.280, 1.249	0.2004
		TT	44	47.8	18	58.1	1.000		
		TA	40	43.5	13	41.9	0.586	0.260, 1.241	0.1769
		AA	8	8.7	0	0.0	0.344	0.068, 1.539	

SNP: Single-nucleotide polymorphism.

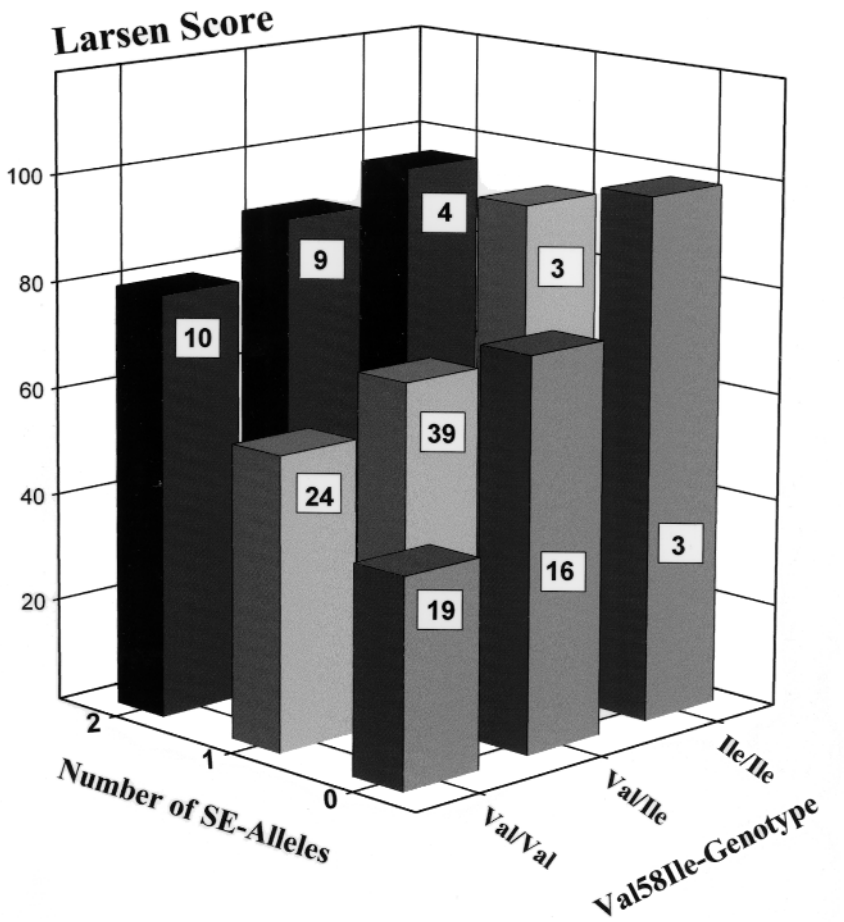

Figure 2. Adjusted Larsen scores of the chondromodulin-II Val58Ile genotypes in subgroups of RA patients with 0, 1, or 2 shared epitope (SE) alleles. Data shown as means. ANOVA for Val58Ile genotypes: 0 SE, p = 0.003; 1 SE, p = 0.186; 2 SE, p = 0.624. ANOVA for SE: ChM-II Val/Val, p = 0.001; Val/Ile, p = 0.107; Ile/Ile, p = 0.732.

Ile/Ile genotype appeared only in haplotype combination TAT/TAT, which was associated with the highest Larsen score values for patients with RA (Table 6). Further, the TAT haplotype in combination with CGA and TGA haplotypes was also associated with increased Larsen scores (Table 6). In combination with the TGT haplotype, however, the TAT haplotype resulted in median Larsen scores. Nevertheless, the homozygous TAT haplotype and the TAT haplotype in combination with CGA and TGA haplotypes should be regarded as potential risk factors for accelerated RA-induced joint destruction.

DISCUSSION

Cartilage and bone destruction are key pathophysiological processes determining the progression and outcome of RA. Growth factors, like chondromodulin-II, originating from the cartilage may have a major role in repair reactions after RA-induced cartilage and bone damage. This hypothesis was recently given support in a study from Japan, which described that a particular polymorphism in exon 3, i.e., a G to A change at nucleotide position 172, resulting in a valine to isoleucine substitution at amino acid position 58, was associated with severity of RA in Japanese patients[11]. Analyzing the complete genotype of ChM-II in a clinically well defined German RA population, we were able to demonstrate that the Val58Ile polymorphism was significantly associated with radiographic damage. Since Larsen scores were adjusted for sex, age at disease onset, and disease duration, our data clearly indicate an acclerated progression of joint destruction in RA patients that were homozygous for the Ile58 allele. Further, RA patients carrying the Ile58 allele had at least 3 times higher risk to develop severe radiographic damage. In contrast to the Japanese study, we could not find a higher Ile58 allele frequency in the German RA group in comparison to German patients with OA or gout. Possible reasons for this inconsistency might be the different genetic background and the relatively small number of investigations in the 2 studies.

Our extended analysis of the ChM-II gene revealed 11 single-nucleotide substitutions, including 5 missense muta-

Table 5. Chondromodulin-II haplotypes in patients with RA, OA and gout. Data as number of alleles with percentages in parentheses.

Haplotype	Polymorphism			Group		
	71 T>C Exon 2	172 G>A Exon 3	–36 T>A Intron 3	RA, n = 254 (%)	OA, n = 162 (%)	Gout, n = 232 (%)
1	T	G	T	99 (39.0)	61 (37.7)	91 (39.2)
2	T	A	T	86 (33.9)	52 (32.1)	88 (37.9)
3	T	G	A	53 (20.9)	41 (25.3)	44 (19.0)
4	C	G	A	13 (5.1)	7 (4.3)	9 (3.9)
5	T	A	A	2 (0.8)	1 (0.6)	ND
6	C	G	T	1 (0.4)	ND	ND

ND: not detected.

Table 6. Effect of chondromodulin-II haplotype combinations on Larsen score in patients with RA. Excluded from analysis: TGT/CGA (n = 3), TGA/CGA (n = 1), TGA/TAA (n = 1). Effect for haplotype combinations on Larsen score in univariate analysis of variance with disease duration, presence of SE allele, rheumatoid factor, antinuclear antibodies, and nodules as covariates: p = 0.027.

Haplotype Combination	n	Larsen Score Adjusted Mean	95% CI
TGA/TGA	6	39.6	12.7–66.5
TGT/TGA	23	57.3	43.6–71.1
TGT/TAT	36	61.1	50.2–72.1
TGT/TGT	20	62.6	47.8–77.3
TGA/TAT	22	77.1	63.1–91.2
CGA/TAT	5	78.6	48.7–108.5
TAT/TAT	10	91.1	70.0–112.3

tions. Interestingly, an association between ChM-II genotype and severity of RA could be established only for the Ile58 allele, suggesting a region of high functional importance. Indeed, protein structure prediction (EMBL, Heidelberg, Germany) indicates a possible influence of the Val58Ile polymorphism on a high-quality sheet structure.

The suggested functional implication of a modified protein structure is also supported by haplotype construction. The homozygous Ile58 allele was exclusively present in the TAT/TAT haplotype combination, thereby increasing the probability of a specific protein conformation. Whether the modified protein structure has significant functional consequences remains to be elucidated.

Chondromodulin-II initially was extracted from fetal bovine cartilage[6]. The novel protein stimulated proteoglycan synthesis in rabbit cultured growth plate chondrocytes, and led to expression of a differentiated phenotype of chondrocytes[6]. Interestingly, it stimulated DNA synthesis in chondrocytes in both the absence and the presence of fibroblast growth factor-2[6]. Unlike other cartilage-derived growth factors, ChM-II synergistically stimulates the growth of osteoblasts and osteoclast differentiation[7,8]. When mouse bone cells were cultured on dentin slices, ChM-II stimulated pit formation dose-dependently and caused an increase in the tartrate-resistant acid phosphatase-positive cells[8]. Therefore, ChM-II is both chondromodulin and osteopoietin, and is the first naturally generated osteopoietic coupling of cartilage and bone growth. Further, the protein is highly homologous with the 2 repeating units of the myb-induced myeloid protein 1 (Mim-1) protein recently shown to be secreted by osteoclasts and to induce bone resorption[15].

ChM-II is identical to the leukocyte cell-derived chemotaxin 2 (LECT2). Investigating systemic LECT2 expression in humans, Nagai, *et al*[16] found general expression in liver, vascular, endothelial and smooth muscle cells, adipocytes, and several epithelial cells. Alternatively, this protein was generally negative, although it was occasionally positively stained in osteoblasts, chondrocytes, cardiac and skeletal muscle cells, smooth muscle cells of the gastrointestinal tract, and epithelial cells of some tissues[16]. It is notable that osteoblasts became positive when the affected bone was fractured[16]. The authors concluded that LECT2 seems to be related to the cell cycle or repair process following damage to a variety of cells. In support of this hypothesis, Saito, *et al*[17] recently described exacerbation of concanavalin A-induced hepatitis in LECT2-deficient mice.

The human ChM-II gene was mapped to chromosome 5q31.1-q32 by fluorescence *in situ* hybridization[10]. This region contains a cluster of cytokine genes including IL-4, IL-5, and IL-9 that are potential candidates for inflammatory disease, bone erosion, and cartilage destruction. Thus, one of the markers found to be correlated with RA susceptibility in a genome-wide linkage study was positioned on chromosome 5q32-33, i.e., relatively close to the ChM-II locus[18]. As well, association with RA susceptibility has been reported for a polymorphism in a noncoding region of the IL-4 gene, located in the same chromosomal region[19]. We could not definitively exclude the possibility that the association of the ChM-II Val58Ile polymorphism and severity of RA was caused by some other candidate gene of this chromosomal region. However, although all SNP in the ChM-II gene are at strong linkage disequilibrium, a significant asso-

ciation could only be established for the Val58Ile polymorphism, supporting its role as a candidate gene.

In accord with other studies[20], our data confirm that HLA-DRB1 risk alleles were an important factor predisposing for RA and increasing the progression rate of radiographic damage. The presence of 2 SE alleles in our population resulted in a significantly younger age at disease onset (40.1 versus 49.3 and 46.8 years, respectively, for one or no SE allele) and a significantly higher degree of radiographic joint destruction (Larsen scores 88.9 versus 61.9 and 57.8 for one or no SE allele).

Potentiation of RA-induced joint damage by the co-occurrence of SE and ChM-II 172A alleles could not be verified by statistical means. In a model of univariate ANOVA with HLA status and ChM-II genotype as factors, and sex, age at disease onset, and disease duration as covariates, ChM-II ($p = 0.002$) and SE ($p = 0.042$) exerted significant and independent effects on the Larsen score.

However, after stratification of RA patients for the number of SE alleles they carried, significant effects of ChM-II 58Val/Ile and Ile/Ile genotypes on Larsen scores could only be established for SE-negative individuals. It is thus of great interest that significant effects of SE on Larsen score appeared exclusively in subjects with the ChM-II 58Val/Val genotype. Nonetheless, the co-occurrence of SE and ChM-II risk alleles resulted in a clear tendency for higher Larsen scores.

We hypothesize that co-occurrence of DRB1 SE alleles, which have been associated with higher risk of induction of RA and development of more pronounced erosions, and ChM-II risk alleles, which seem to reduce repair capabilities, might result in a potentiated and accelerated RA-induced joint destruction. Whether genetic interactions between the SE and ChM-II might in fact enhance disease progression should be reanalyzed by an extended study.

At present, the pathophysiological role of ChM-II in RA-induced joint destruction is unknown. ChM-II may modulate cartilage and bone metabolism as well as inflammatory and immune responses induced by the development of RA. The ChM-II polymorphisms we tested were in strong linkage disequilibrium and might affect levels of gene expression, resulting in differential expression of ChM-II protein. It is tempting to speculate that an abnormal level of ChM-II expression might disrupt communication between chondrocytes, osteoblasts, and osteoclasts, resulting in cartilage and bone destruction. On the other hand, the ChM-II Val58Ile polymorphism might result in a modified protein conformation leading to altered downstream effects. However, any downstream functional partners have not been identified yet.

A study of transgenic mice lacking the ChM-II (LECT2) gene revealed an increasing number of natural killer (NK) T cells in the liver[17]. It is well established that NK T cells express large amounts of cytokines, especially IL-4 and interferon-γ[21]. IL-4 has been characterized as an anticatabolic cytokine in inflammatory joint processes, inhibiting cartilage-degrading proteinases and decreasing the production of many of the catabolic and proinflammatory cytokines by synovial fibroblasts or chondrocytes[22]. It could be hypothesized that a modified ChM-II protein is accompanied by an increasing number of NK T cells accumulating in inflamed joints, with subsequent production of IL-4 and other antiinflammatory cytokines.

ChM-II has been shown to be constitutively expressed in liver, whereas in mature bone it was detectable only after bone fracture[16]. Factors involved in ChM-II gene expression remain to be identified. In a recent study, Ovejero, *et al*[23] described ChM-II (LECT2) as a target gene of the WNT/ß-catenin signaling pathway. By mutagenesis of the ChM-II promoter, a binding site for the LEF/TCF transcription factors could be identified as being crucial for ChM-II activation by ß-catenin[23]. Recent observation that the canonical WNT signaling pathway plays a central role in regulating many aspects of skeletal development, from limb formation to chondrogenesis and osteoblast maturation, is a major advance in understanding skeletal biology[24]. Mutations in genes encoding WNT signal proteins are now linked to inherited human skeletal disorders like osteoporosis pseudoglioma syndrome (LRP5) and high bone mass disorders (LRP5)[24]. Further, glucocorticoids, which are known to cause osteoporosis by inhibiting bone formation, inhibited the LEF/TCF transcriptional activity in differentiating osteoblasts[25]. Thus, ChM-II belongs to a group of downstream targets of the WNT/ß-catenin signaling pathway that promotes proliferation, expansion, and survival of osteoblasts, chondrocytes, and perhaps osteoclasts, and might play a crucial role in repair processes.

Future studies should address the identification of downstream targets of ChM-II that are involved in chondrocyte, osteoblast, and osteoclast signaling pathways in order to differentiate whether the effects of ChM-II are preferentially mediated through expression level or protein conformation. Well characterized animal models (e.g., SCID mouse), which have been widely used to elucidate the pathogenesis of RA, seem to be well suited for studying the role of ChM-II and other growth factors in the modulation of repair processes in rheumatoid joint destruction.

Our study of clinically well defined German patients with RA revealed that the Val58Ile polymorphism in exon 3 of the chondromodulin-II gene was significantly associated with a higher risk for accelerated progression of radiographic joint destruction, and this effect was modified by the HLA status.

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