Association Between Interleukin 1 Gene Cluster Polymorphisms and Bilateral Distal Interphalangeal Osteoarthritis

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ABSTRACT. ***Objective.*** To examine the association of the interleukin 1 gene (*IL1*) cluster polymorphisms and their haplotypes with bilateral distal interphalangeal joint osteoarthritis (DIP OA).

Methods. Radiographs of both hands of 295 dentists and 248 teachers were examined and classified for the presence of OA using reference images. Bilateral DIP OA was defined by the presence of radiographic findings of grade 2 or more in at least 1 symmetrical pair of the DIP joints. We genotyped 10 single-nucleotide polymorphisms (SNP) in the *IL1R1*, *IL1RL2*, *IL1A*, *IL1B*, and *IL1RN* genes using polymerase chain reaction-based methods. Haplotypes were statistically reconstructed using the PHASE program. The association between the genotypes/diplotypes and bilateral DIP OA was examined with logistic regression analysis.

Results. Two *IL1B* SNP (rs1143634 and rs1143633) were associated with bilateral DIP OA. The carriers of the *IL1B* rs1143634 minor allele had an increased OA risk [odds ratio (OR) 1.6; 95% confidence interval (CI) 1.08–2.26] compared to the noncarriers. The association was stronger in the dentists. The distribution of the *IL1B* rs1143633 genotype fit a recessive mode of inheritance (OR 3.03, 95% CI 1.35–6.83, p = 0.006). Two *IL1B-IL1RN* extended haplotype alleles (211-1 and 121-1) were associated with bilateral DIP OA. An interaction between the *IL1B* rs1143634 and the *IL1R1-IL1RL2* and *IL1B-IL1RN* extended haplotypes and occupation (increased risk of OA among dentists only) was observed.

Conclusion. Our results provide further evidence for the role of *IL1* gene cluster polymorphisms in the etiology of OA and suggest that some of these may predispose DIP joints to the effects of mechanical overload. (First Release August 15 2009; J Rheumatol 2009;36:1977–86; doi:10.3899/jrheum.081238)

Key Indexing Terms:
HAND BILATERAL OSTEOARTHRITIS DISTAL INTERPHALANGEAL JOINT INTERLEUKIN 1 GENE CLUSTER POLYMORPHISMS

Osteoarthritis (OA) is the most common joint disease and is among the most frequent health problems in the general population[1]. Risk factors of hand OA include age, sex, acute injury, repetitive joint loading, and obesity[2].

Published data suggest that genetic factors play a major role in the etiology of OA[3]. Genetic susceptibility may be more relevant to OA in women than in men and may differ between joint groups[4,5]. Leppävuori, *et al*[6] found evidence

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of linkage of distal interphalangeal (DIP) joint OA with the chromosomal region on 2q12-q14 harboring the interleukin 1 gene (*IL1*) cluster.

The *IL1* gene cluster includes genes coding for IL-1α (*IL1A* on 2q13), IL-1ß (*IL1B* on 2q13), IL-1 type I receptor (*IL1R1* on 2q12), IL-1 receptor-like 2 protein (*IL1RL2* on 2q12), IL-1 type II receptor (*IL1R2* on 2q12-q22), and IL-1 receptor antagonist (*IL1RN* on 2q14.2). IL-1α, IL-1ß, and IL receptor antagonist (IL-1Ra) are structurally related to one another and act by directly binding to IL-1 receptors[7]. Both IL-1α and IL-1ß are strong inducers of inflammation and recognize the same receptor, IL-1RI[8]. By contrast, IL-1Ra acts as an inhibitor of IL-1 activity.

In vivo animal models of arthritis have shown that either local overproduction of IL-1ß and/or underproduction of IL-1Ra may predispose to local tissue destruction[9]. IL-1 stimulates the synthesis and activity of matrix metalloproteinases and other enzymes involved in cartilage destruction in rheumatoid arthritis and OA[10].

All *IL1* cluster genes are highly polymorphic. A single-nucleotide polymorphism (SNP) in the promoter region ($C^{-889}T$; rs1800587) of the *IL1A* gene[11], SNP in the promoter region ($C^{-511}T$; rs16944) and in exon 5 ($C^{+3954}T$; rs1143634) of the *IL1B* gene[12,13], and a variable-number tandem repeat (VNTR) polymorphism in the intron 2 of the *IL1RN* gene[14-16] have biological relevance in the regulation of IL-1 and IL-1Ra production. Plasma concentrations of IL-1Ra may be coordinately regulated by both *IL1RN* and *IL1B* genes[12].

Associations of both single-locus polymorphisms and extended haplotypes within the *IL1* gene cluster with hip, knee, and erosive hand OA have been observed in some studies[17-22], while others have reported lack of association[23,24]. Various factors may have contributed to these apparently discordant results among studies, including differences in the *IL1* genotype distributions, and, especially, failure to take into account factors that modulate the effect of *IL1* gene cluster on the risk of OA. Uchida, *et al*[25] proposed that either excess or absence of mechanical force may induce the secretion of cytokines, such as IL-1ß. Recent studies showed that moderate load inhibits the IL-1ß, while its expression is upregulated with high loads[26,27].

Given the suggestive linkage and association evidence of certain OA phenotypes with the *IL1* gene loci, the purpose of our study was to examine whether the functionally relevant *IL1* gene cluster polymorphisms or their haplotypes are associated with bilateral DIP OA among samples of Finnish women from 2 occupations. Further, we evaluated whether the association of DIP OA with the *IL1* gene cluster polymorphisms varies by the differential use of the hands. Assuming that hand usage among dentists is characterized by stereotyped repetitive tasks for prolonged periods of time, we expected that the association of OA with the *IL1* gene cluster polymorphisms is more evident among dentists than among women from an occupation within the same socioeconomic grade but with lower hand load (teachers).

MATERIALS AND METHODS

Subjects. Potential study subjects were identified through the registers of the Finnish Dental Association and the Finnish Teachers' Trade Union. We randomly selected 436 women aged 45–63 years from each occupational group. The place of residence was an inclusion criterion (Helsinki or its neighboring cities) for participation in a study concerning work-related factors and individual susceptibility in the etiology of hand OA. Of those who received the questionnaires in 2002, 295 (67.7%) dentists and 248 (56.9%) teachers participated in a clinical examination. Participation in the study was voluntary and based on informed consent. The Hospital District of Helsinki and Uusimaa Ethics Committee for Research in Occupational Health and Safety approved the study proposal.

Bilateral DIP OA. Both hands of the participants were radiographed by Kodak radiographic films exposed with Siemens radiographic equipment (48 kV, 10 mAs, focus–film distance 115 cm). The analog radiographs were evaluated by an experienced radiologist blinded to the occupation, age, and all health data of the subjects.

Each DIP joint of both hands was graded separately, and classified for the presence of OA using a modified Kellgren and Lawrence system. The classification criteria were: grade 0 = no OA; grade 1 = doubtful OA; grade 2 = mild OA; grade 3 = moderate OA; grade 4 = severe OA. Reference images were used. The description of the reference images has been given elsewhere[28]. The reliability of the readings was estimated by measuring intra- and interobserver agreement, using the weighted Cohen's kappa coefficient with quadratic weights, for 46 randomly chosen subjects. For this, a second reading was independently performed by TV and another experienced radiologist (KL). The interobserver agreement for DIP OA ranged from 0.67 to 0.85. Inter- and intraobserver agreements (range 0.67–0.85 and 0.73–0.88, respectively) indicated good reliability. Only the readings of TV were used in the subsequent statistical analyses.

If the subject had radiographic findings (grade ≥ 2) in at least 1 symmetrical pair of the DIP joints (if 1 DIP joint of the hand is affected, the same joint of the opposite hand is also affected), she was classified as having bilateral DIP OA.

Genotyping analysis. All DNA samples were extracted from lymphocytes by DNA extraction kit (PureGene DNA Purification Kit, Gentra, Minneapolis, MN, USA).

The *IL1A* $C^{-889}T$ (rs1800587) genotypes were resolved by a polymerase chain reaction (PCR)-based method employing primers described by McDowell, *et al*[29]. Briefly, the downstream primer contains a nucleotide change that generates in the PCR an additional *NcoI* restriction site in the wild-type allele. Aliquots of the PCR products were digested with *NcoI* restriction enzyme (New England Biolabs, Beverly, MA, USA) and electrophoresed on 4% agarose gel (Cambrex, East Rutherford, NJ). The final results were interpreted from pictures of the gels photographed under ultraviolet light; the allele with the restriction site for *NcoI* was denoted as *1A1* (C) and the allele lacking the site as *1A2* (T).

The *IL1B* genotypes were determined using PCR-based methods essentially as described. Briefly, the *IL1B* $C^{-511}T$ (rs16944), $C^{+3954}T$ (rs1143634), and *IL1B* $G^{+5810}A$ (rs1143633) base changes

were identified by *Ava*I, *Taq*I, and *Xcm*I restriction enzyme digestions subsequent to PCR amplification, respectively[30,31].

The polymorphic region of the *IL1RN* gene containing a VNTR is situated in the second intron of the gene. The *IL1RN* VNTR polymorphism was analyzed by PCR using the following primers: 5' CTC AGC AAC ACT CCT AT 3' (sense); and 5' TCC TGG TCT GCA GGT AA 3' (antisense) as described[32]. The number of 86-bp long repeats was detected on a standard 2% agarose gel stained with 0.1% ethidium bromide. The detected PCR products were 412 bp (allele 1 = 4 repeats of the 86 bp region), 240 bp (allele 2 = 2 repeats), 326 bp (allele 3 = 3 repeats), and 498 bp (allele 4 = 5 repeats) in length.

The genotyping of *IL1R1* (rs1465325, rs956730, rs2287047) and *IL1RL2* (rs1922290, rs1922295) SNP was performed using a single-base extension fluorescent method, the SNaPshot Multiplex System (Applied Biosystems). PCR amplification and SNaPshot reactions were designed to have annealing temperatures of 60°C and 55°C, respectively. The extension primer was designed to anneal immediately adjacent to the nucleotide at the polymorphic site. Multiplex-PCR was carried out in a total volume of 15 µl containing 60 ng DNA, 0.15–0.35 µM of each primer, 0.25 mM of each dNTP, 2.5 U Hot Start Taq DNA polymerase (Fermentas), and 25 mM $MgCl_2$. PCR products were purified with SAP/ExoI treatment to remove primers and unincorporated deoxyribonucleotide triphosphate. The purified product was then processed according to the SNaPshot Multiplex System protocol (Applied Biosystems, Foster City, CA, USA). Briefly, the SNaPshot reaction contained 3 µl of purified PCR product, 0.15–0.5 µM extension primers, and 5 µl SNaPshot Multiplex Ready Reaction Mix in a final volume of 10 µl. The reaction was then purified using 1 U of SAP at 37°C for 1 h followed by incubation at 75°C for 15 min. Analysis was carried out with the ABI Prism 3100 genetic analyzer (Applied Biosystems) and genotypes were determined using the GeneMapper software (Applied Biosystems).

To ensure laboratory quality control in genotyping tests, 2 independent readers interpreted the results. In every genotyping analysis, internal control samples with known genotypes were included. Any sample with ambiguous results was retested, and a random selection of 10% of all samples was repeated. No discrepancies were discovered upon replicate testing within the method used. For technical reasons, 7 of the 543 samples could not be genotyped for the *IL1R1*, *IL1RL2*, and *IL1RN* polymorphism.

Other risk factors. The self-administered questionnaire included items on anthropometric measures and smoking history.

Six main tasks in dental work were identified prior to the study: restorative treatment and endodontics, orthodontics, periodontics, prosthodontics, surgical treatment, and other nontreatment activities (e.g., dental examination, consulting, and administrative tasks). The subjects were asked to recall their work history in 10-year periods (at the age of 25–34, 35–44, and 45–54 yrs) in terms of average number of working hours per week, and the proportion of time (percentage) performing each task during an average working day. Based on the weekly hours of the work tasks, dental task variation was empirically defined using cluster analysis with the K-means algorithm[33]. Three clusters were identified: cluster 1 (high variation) consisted of 96 (33%) dentists who performed a combination of different tasks, cluster 2 (moderate variation) of 64 (22%) dentists who spent about half their work time on restoration treatment and endodontics and another half on prosthodontics, periodontics and surgical treatment, and cluster 3 (low variation) of 131 (45%) dentists mainly performing restoration treatment and endodontics.

Weight was measured without shoes to the accuracy of 0.1 kg. Body mass index (BMI; weight per height squared) was calculated based on self-reported height and measured weight. BMI was dichotomized for logistic regression analysis (normal weight BMI < 25.0 kg/m^2; overweight or obese BMI ≥ 25.0 kg/m^2). Based on their smoking history the subjects were classified into never daily smokers or ever (current or previous) smokers.

Statistical analyses. The potential deviation from the Hardy-Weinberg equilibrium (HWE) was tested using the chi-squared test. First, each gene locus was investigated separately. The allele and genotype frequencies were compared between individuals with and without OA using Fisher's exact probability test or the chi-squared test. We tested a series of genetic models (dominant, additive, and recessive) for estimation of best fit for the risk of OA[34]. The SNPSpD method[35] was used to calculate the p value threshold for 5% significance.

The degree of pairwise linkage disequilibrium (LD) was calculated for each pair of SNP using Haploview software[36]. Because the loci were in close proximity to each other, a haplotype analysis was also performed to investigate whether the underlying LD contributed to the nonindependence of these associations. The haplotypes were statistically reconstructed from population genotype data using the PHASE program with the Markov-chain method for haplotype assignments[37]. Haplotypes with an observed frequency of less than 0.05 were analyzed combined as a group. To identify potential risk or protective haplotypes, haplotype frequency comparisons between subjects with and without bilateral DIP OA were performed using Fisher's exact probability test or the chi-squared test.

We performed a set of hierarchical logistic regression analyses to examine the association between the *IL1* gene cluster and bilateral DIP OA. Baseline models included only the polymorphism as the predictor. Additional models were tested with age (in years) and occupation (dentists vs teachers), and final models also with BMI (normal vs overweight) and smoking history (never vs ever) as covariates. Crude and adjusted odds ratios (OR) and their 95% confidence intervals (CI) were calculated using the SPSS statistical package.

Interactions between haplotypes and occupation were investigated by stratification and by logistic regression analysis. To evaluate the interaction between the SNP and the variation in dental work tasks, the risk of OA was calculated as a function of variation in dental tasks (low task variation or moderate task variation or high variation of dental tasks), of the presence of a risk allele, and of their combination. The absence of the risk allele and high variation of tasks was used as the reference group.

Because the haplotype and genotype analyses are not entirely independent tests, p values were not corrected for multiple testing. The statistical significance of the p value was defined as the 5% level.

RESULTS

The prevalence of bilateral DIP OA was 38% (46% in teachers and 31% in dentists; p = 0.0001). Subjects with OA were significantly older and had higher BMI than those without

OA (55.7 ± 4.8 vs 52.8 ± 5.2 years, p = 0.0001; and BMI 25.0 ± 3.8 vs 24.1 ± 3.4, p = 0.01, respectively).

We genotyped 3 SNP in the long promoter and the coding region of gene *IL1R1* (rs1465325, rs956730, rs2287047), 2 SNP in the coding region of gene *IL1RL2* (rs1922290, rs1922295), 1 in the promoter region of *IL1A* gene, 3 SNP in the *IL1B* gene (rs16944 in promoter region, rs1143634 in exon 5, and rs1143633 in intron 4), and a VNTR polymorphism in the intron 2 of *IL1RN* gene. Genotype frequencies for all SNP except rs1143633 (in subjects without DIP OA, p = 0.03) were in HWE. Four alleles for the *IL1RN* VNTR polymorphism were identified. Allele 1 (4 repeated units) had a frequency of 0.73, allele 2 (2 repeats) a frequency of 0.25, allele 3 (3 repeats) a frequency of 0.002, and allele 4 (5 repeats) a frequency of 0.001. The frequency of alleles, genotypes, and carriage rates of the polymorphisms did not statistically significantly differ between the occupational groups.

Association of the IL1R1, IL1RL2, IL1A, IL1B, and IL1RN VNTR polymorphisms with bilateral DIP OA. According to SNPSpD, the effective number of independent SNP loci was 4.2, thus the experiment-wide significance threshold of 0.011 was required to keep the type I error rate at 5%. Two *IL1B* polymorphisms (rs1143634 and rs1143633) were associated with bilateral DIP OA (Table 1). The distribution of the *IL1B* rs1143634 genotype fitted dominant and additive modes of inheritance (OR 1.56, 95% CI 1.08–2.26, and OR 1.41, 95% CI 1.05–1.88, respectively), but the associations were not statistically significant after correction for multiple testing. The distribution of the *IL1B* rs1143633

Table 1. Association of the *IL1* gene cluster polymorphisms with bilateral DIP OA.

SNP	Genotype	Counts (No/Yes)	OR† (95% CI)	p* General Association	Dominant	Additive	Recessive
IL1R1							
rs1465325	T/T	204/124	1	0.42	0.39	0.25	0.24
	T/C	115/72	1.13 (0.76–1.66)				
	C/C	9/10	1.86 (0.70–4.93)				
rs956730	G/G	164/94	1	0.51	0.30	0.47	0.82
	G/A	138/97	1.25 (0.85–1.84)				
	A/A	26/15	1.02 (0.50–2.12)				
rs2287047	C/C	168/103	1	0.79	0.49	0.51	0.78
	C/T	135/87	1.13 (0.77–1.67)				
	T/T	25/16	1.17 (0.57–2.40)				
IL1RL2							
rs1922290	G/G	138/82	1	0.42	0.53	0.99	0.40
	G/C	128/90	1.24 (0.82–1.86)				
	C/C	62/34	0.90 (0.53–1.53)				
rs1922295	G/G	137/82	1	0.45	0.56	0.99	0.39
	G/A	145/98	1.20 (0.81–1.79)				
	A/A	46/26	0.87 (0.48–1.56)				
IL1A							
rs1800587	C/C	149/91	1	0.63	0.59	0.90	0.56
	C/T	145/95	1.16 (0.79–1.71)				
	T/T	41/21	0.90 (0.48–1.68)				
IL1B							
rs1143634	C/C	189/100	1	0.06	0.02	0.02	0.27
	C/T	123/88	1.53 (1.03–2.25)				
	T/T	23/19	1.76 (0.87–3.55)				
rs1143633	G/G	170/118	1	0.001	0.13	0.88	0.006
	G/A	154/69	0.62 (0.42–0.92)				
	A/A	11/20	2.48 (1.08–5.68)				
rs16944	C/C	121/85	1	0.48	0.24	0.37	0.91
	C/T	167/92	0.78 (0.52–1.16)				
	T/T	47/30	0.85 (0.48–1.50)				
IL1RN VNTR	1/1	185/114	1.00	0.14	0.86	0.54	0.08
	1/2	110/77	1.20 (0.80–1.79)				
	2/2	35/14	0.58 (0.28–1.18)				

* Noncorrected p value of the association analysis. † Adjusted for age, occupation, BMI, and smoking history. For the *IL1RN* variable-number tandem repeat (VNTR) polymorphism, allele 1 consists of the alleles with 4 repeat units, 3 repeat units, and 5 repeat units, allele 2 has 2 repeat units. DIP OA: distal interphalangeal osteoarthritis; BMI: body mass index.

genotype fitted a recessive mode of inheritance (OR 3.03, 95% CI 1.35–6.83) and remained statistically significant after correction for multiple testing. No statistically significant association of the *IL1R1*, *IL1RL2*, *IL1A*, and *IL1B* rs16944 SNP and the *IL1RN* VNTR polymorphism with bilateral DIP OA was found.

Seventy-six women had unilateral DIP OA out of 328 women without bilateral DIP OA. The association between the polymorphisms and bilateral DIP OA was not altered when the analysis was repeated with a more strictly defined control group, i.e., removing subjects with the unilateral DIP OA. No relationship between the polymorphisms and unilateral DIP OA was found (data not shown).

Stratification by occupation showed a difference in the magnitude of the association of the *IL1B* rs1143634 SNP with OA between dentists and teachers. The carriage of the rs1143634 minor allele was associated with an increased risk of OA among the dentists only (dentists: OR 1.92, 95% CI 1.11–3.31, p = 0.02; teachers: OR 1.35, 95% CI 0.80–2.26, p = 0.26).

To examine the possible interaction between the *IL1B* polymorphisms and occupational hand load, we estimated the main and joint effects of the polymorphisms and the type of work history among the dentists. We found that both low variation in work tasks and the carriage of the rs1143634 minor allele had an effect on the risk of OA (Table 2, OR 3.13, 95% CI 1.11–8.85, p = 0.03 and OR 4.44, 95% 1.47–13.43, p = 0.008, respectively) and when the factors occur together, the risk of OA was higher (OR 4.84, 95% 1.66–14.11, p = 0.004) than when they occur alone.

Linkage disequilibrium in the IL1R1-IL1RL2-IL1A-IL1B region. A LD plot for the SNP studied here is presented in Figure 1. Haploview analysis showed that in our data the degree of LD (measured as D') was high within the genes. There was considerable intergenic LD between SNP in the *IL1R1* and *IL1RL2* genes. The LD between the *IL1A-IL1B* and the *IL1R1-IL1RL2* regions was very low.

Because of the low frequency of the alleles 3 and 4, the VNTR polymorphism was considered biallelic (4 repeats vs other). The LD for the *IL1RN* VNTR polymorphism with either *IL1A* or *IL1B* SNP was statistically significant (D' varied from 0.34 to 0.62). The degree of LD for the VNTR polymorphism with the *IL1R1* or *IL1RL2* SNP was weak (D' varied from 0.03 to 0.07).

Association of the IL1R1-IL1RL2 and IL1A-IL1B-IL1RN extended haplotypes with DIP OA. Because of an overall weak intergenic LD between the *IL1R1-IL1RL2* and the *IL1A-IL1B-IL1RN* regions, and relatively low LD between the rs180057 (*IL1A*) and other polymorphisms within the *IL1B-IL1RN* region, separate haplotype analyses were conducted for the *IL1R1-IL1RL2* and the *IL1B-IL1RN* region. The frequencies of haplotype alleles for the *IL1R1-IL1RL2* and *IL1B-IL1RN* in subjects with and without bilateral DIP OA are presented in Table 3.

The rs956730 and rs2287047 SNP (*IL1R1*) and rs1922290 and rs1922295 SNP (*IL1RL2*) were in complete linkage, therefore the *IL1R1-IL1RL2* haplotypes were reconstructed based on the genotypes at 3 loci. Of the 7 haplotypes derived from the analysis, 4 were common. There was no difference between subjects with and those without bilateral DIP OA in the *IL1R1-IL1RL2* haplotype distribution. However, a statistically significant (p = 0.02) interaction between the *IL1R1-IL1RL2* haplotypes and occupation was observed. The *IL1R1-IL1RL2* 112 haplotype was associated with an increased risk of OA among the dentists (Table 4; OR 1.75, 95% CI 1.15–2.68, p = 0.009) and a reduced risk of OA among the teachers (OR 0.66, 95% CI 0.43–1.01, p = 0.055). When we examined the possible interaction between the haplotype and occupational hand load among the dentists, we found that the haplotype exacerbates the effect of low variation in dental work tasks on the risk of OA, but there was no effect of the haplotype in dentists with high variation in work tasks (dentists with low variation in work tasks and the carriers of the haplotype: OR 2.25, 95% CI 0.91–5.54, p = 0.08; and dentists with high variation in work tasks and noncarriers of the haplotype: OR 0.73, 95% CI 0.28–2.01, p = 0.54).

Based on the genotypes at the 4 polymorphic loci a total of 16 *IL1B-IL1RN* VNTR haplotypes were reconstructed. Five common haplotypes were identified. Overall, the

Table 2. Main and joint effect of the *IL1B* rs1143634 minor alleles and variation in dental work tasks on bilateral DIP OA.

Carriage of Variant Allele	Level of Variation in Work Tasks	Counts (No/Yes)	OR (955 CI)†	p
No	High	40/6	1.0	
No	Moderate	22/9	1.90 (0.55–6.55)	0.31
No	Low	48/24	3.13 (1.11–8.85)	0.03
Yes	High	33/17	4.44 (1.47–13.43)	0.008
Yes	Moderate	23/10	2.92 (0.87–9.73)	0.08
Yes	Low	33/26	4.84 (1.66–14.11)	0.004

† Adjusted for age, BMI, level of leisure time hand-loading physical activity, and smoking history. For abbreviations, see Table 1.

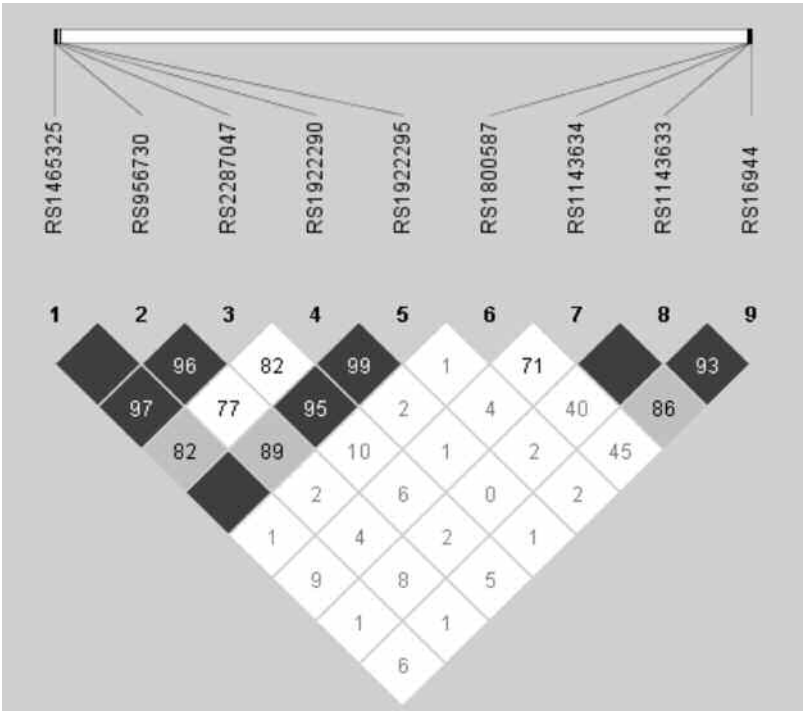

Figure 1. Haploview linkage disequilibrium plot of the *IL1R1* (rs1465325, rs956730, rs2287047), *IL1RL2* (rs1922290, rs1922295), *IL1A* (rs1800587), and *IL1B* (rs1143634, rs1143633, rs16944) single-nucleotide polymorphisms.

Table 3. Extended haplotype frequency distribution according to bilateral DIP OA status.

		Bilateral DIP OA					
		No (n = 660–670)		Yes (n = 410–414)			
Gene	Haplotype	N	Frequency	N	Frequency	OR (95% CI)†	p
IL1R1–	H1: 111	237	0.36	139	0.34	1.00	0.69***
*IL1RL2**	H2: 112	231	0.35	148	0.36	1.07 (0.79–1.45)	0.696
	H3: 221	132	0.20	90	0.22	1.22 (0.85–1.74)	0.320
	H4: 121	53	0.08	29	0.07	0.98 (0.57–1.86)	0.866
	H5: rare	7	0.01	4	0.01	0.51 (0.08–3.04)	0.525
*IL1B-IL1RN***	H1: 112–1	118	0.18	66	0.16	1.00	0.46***
	H2: 211–1	149	0.23	114	0.28	1.52 (1.01–2.28)	0.044
	H3: 121–1	136	0.21	87	0.21	1.17 (0.76–1.79)	0.437
	H4: 112–2	125	0.19	76	0.18	1.12 (0.73–1.74)	0.662
	H5: 111–1	70	0.11	35	0.08	0.94 (0.55–1.60)	0.738
	H6: rare	60	0.09	34	0.08	1.04 (0.60–1.79)	0.982

† Adjusted for age, occupation, BMI, and smoking history. For the *IL1R1, IL1RL2, IL1A*, and *IL1B* polymorphisms 1 = common allele, 2 = rare allele. For the *IL1RN* variable-number tandem repeat (VNTR) polymorphism, allele 1 has 4 repeat units; allele 2 has 2 repeat units. * Allele positions were ordered as follows: rs1465325, rs2287047, rs1922295. ** Allele positions were ordered as follows: rs1143634, rs1143633, rs16944, and *IL1RN* VNTR. *** p for global association. For abbreviations, see Table 1.

IL1B-IL1RN haplotype allele frequencies did not differ significantly between subjects with and without DIP OA. However, the distribution of the 211-1 diplotypes fitted an additive mode and the distribution of the 121-1 diplotypes fitted a recessive mode of inheritance (OR 1.42, 95% CI 1.05–1.94, p = 0.025 and OR 4.36, 95% CI 1.48–12.83, p = 0.007, respectively).

A statistically significant (p = 0.01) interaction between the *IL1B-IL1RN* extended haplotype and occupation was observed. The 211-1 haplotype was associated with an increased risk of OA among the dentists only (Table 4; dentists: OR 2.39, 95% CI 1.30–4.41, p = 0.005; and teachers: OR 1.01, 95% CI 0.57–1.79, p = 0.98). The 111-1 haplotype was associated with a reduced risk of OA among the teach-

Table 4. The *IL1R1–IL1RL2* and *IL1B–IL1RN* extended haplotype frequency distribution according to bilateral DIP OA status by occupation.

	Dentists						Teachers					
	No Bilateral DIP OA		Bilateral DIP OA				No Bilateral DIP OA		Bilateral DIP OA			
Haplotype	N	Frequency	N	Frequency	OR (95% CI)†	p	N	Frequency	N	Frequency	OR (95% CI)†	p
*IL1R1–IL1RL2**						0.144***						0.336***
H1: 111	149	0.38	51	0.28	1.00		86	0.33	90	0.39	1.00	
H2: 112	130	0.33	78	0.42	1.75 (1.15–2.68)	0.009	104	0.40	72	0.32	0.66 (0.43–1.01)	0.055
H3: 221	82	0.21	41	0.22	1.48 (0.90–2.42)	0.119	49	0.19	49	0.22	0.96 (0.58–1.59)	0.857
H4: 121	30	0.07	14	0.08	1.36 (0.67–2.77)	0.392	21	0.08	15	0.07	0.68 (0.33–1.41)	0.302
H5: rare	3	0.01	0	0.00	—		2	0.01	2	0.01	0.96 (0.13–6.94)	0.964
*IL1B-IL1RN***						0.032***						0.163***
H1: 112–1	73	0.18	23	0.12	1.00		45	0.17	43	0.19	1.00	
H2: 211–1	94	0.24	62	0.34	2.39 (1.30–4.41)	0.005	55	0.21	52	0.23	1.01 (0.57–1.79)	0.98
H3: 121–1	78	0.19	34	0.18	1.53 (0.79–2.97)	0.212	60	0.23	53	0.23	0.93 (0.53–1.64)	0.869
H4: 112–2	82	0.21	27	0.15	1.10 (0.55–2.16)	0.814	43	0.16	49	0.21	1.20 (0.66–2.18)	0.563
H5: 111–1	36	0.09	21	0.11	2.23 (1.04–4.80)	0.044	34	0.13	14	0.06	0.43 (0.20–0.93)	0.027
H6: rare	35	0.09	17	0.09	1.54 (0.69–3.41)	0.309	25	0.09	17	0.07	0.73 (0.34–1.55)	0.411

† Adjusted for age, BMI, and smoking history. For *IL1R1, IL1RL2*, and *IL1B* polymorphisms 1 = common allele, 2 = rare allele. For the *IL1RN* variable-number tandem repeat (VNTR) polymorphism, allele 1 has 4 repeat units; allele 2 has 2 repeat units. * Allele positions were ordered as follows: rs1465325, rs2287047, rs1922295. ** Allele positions were ordered as follows: rs1800587, rs1143634, rs1143633, rs16944, and *IL1RN* VNTR. p for global association. For abbreviations, see Table 1.

ers (OR 0.43, 95% CI 0.20–0.93, p = 0.027) and an increased risk among the dentists (OR 2.23, 95% CI 1.04–4.80, p = 0.044).

Similar to the results of the low variation of dental work tasks — the *IL1B* rs1143634 interaction analysis, both low variation in work tasks and the carriage of the 211-1 haplotype had an effect on the risk of OA (OR 3.45, 95% CI 1.22–9.74, p = 0.02 and OR 5.69, 95% 1.86–17.42, p = 0.002, respectively). However, the combined effect of these 2 factors on the risk of OA was similar to the effect of the haplotype on the disease risk (OR 5.63, 95% 1.93–16.46, p = 0.002).

DISCUSSION

We found that carriage of the minor alleles of 2 *IL1B* polymorphisms (rs1143633 and rs1143634) and *IL1B-IL1RN* extended haplotypes (211-1 and 121-1) was associated with an increased risk of bilateral DIP OA in middle-aged Finnish women. The genotype distribution for *IL1B* rs1143633 fitted a recessive mode and the *IL1B* rs1143634 a dominant mode of inheritance. In addition, we observed a statistically significant interaction between both the *IL1R1-IL1RL2* and *IL1B-IL1RN* extended haplotypes and occupation.

OA is often defined as a noninflammatory arthropathy. However, there is much evidence to support the view that inflammatory components are involved in the disease process[38]. IL-1α, IL-1ß, and tumor necrosis factor-α have been detected in the synovial fluid and cartilage of patients with OA[39-41]. Cartilage from an OA joint spontaneously produces a higher amount of IL-ß compared with normal cartilage[42]. Among patients with OA at multiple sites (hand, knee, hip, and spine) the innate *ex vivo* production of both IL-1ß and IL-Ra was higher than among controls[43]. The *IL1R1* and *IL1RL2* genes encode IL-1 receptors that have been shown to mediate the activation of nuclear factor-κB, which in turn participates in the regulation of inflammatory and immune-response gene expression[44].

Various linkage studies have suggested a direct effect of the *IL1* gene cluster on OA and associations with different OA phenotypes have been reported. Studies that evaluated single polymorphic markers found an association of the *IL1B* rs16944 minor allele and *IL1RN* VNTR allele 2 with increased risk of hip OA[20]. The *IL1B* rs1143634 minor allele was associated with increased risk of end-stage symptomatic knee or hip OA in 1 study[17] and reduced risk of hip OA in another study[20]. Stern and colleagues[19] found an elevated risk of erosive hand OA in subjects homozygous for the *IL1BA* rs1143633 minor allele. We confirmed the association of the *IL1B* rs1143633 minor allele with another hand OA phenotype (bilateral DIP OA). Further, in our study the *IL1B* rs1143634 minor allele that has previously been associated with high IL-1ß production[12] conferred a 1.5-fold increased risk of bilateral DIP OA. Hand and knee OA aggregate together much more often than does either type with hip OA[45]. Loughlin has suggested that the role of genetic variations in the development and progression of OA may vary between joint groups[5]. Therefore, it is possible that *IL1B* rs1143634 minor allele predisposes to 1 OA phenotype and is protective to another.

Significance of haplotype. Relatively strong LD in the *IL1* gene cluster has been reported[18,20,46]. Previous research has suggested that within the *IL1* gene cluster there might be a haplotype associated with OA, while the individual polymorphisms did not show any association[21]. Two haplotypes

(11-2 and 12-1) based on the *IL1B* rs1143634, rs16944, and *IL1RN* VNTR polymorphisms were found to be associated with hip OA[20,47]. However, this association was not replicated in a larger study by Chapman and Loughlin[23]. Smith and colleagues identified an 8-marker *IL1A-IL1B-IL1RN* extended haplotype predisposing to knee and hip OA[21,47], and a protective *IL1B-IL1RN* haplotype that conferred a 5-fold reduced risk of knee OA[21]. Recently, an association of an *IL1B* haplotype and an *IL1A-IL1B-IL1RN* extended haplotype with hand OA was reported[22]. By constructing 4-marker haplotypes we identified 2 risk haplotypes (211-1 and 121-1) for bilateral DIP OA. The first haplotype was composed of the *IL1B* rs1143634 minor allele and 3 major alleles (*IL1B* rs1143633, *IL1B* rs16944, and *IL1RN* VNTR allele 1). The effect of this haplotype on bilateral DIP OA was not different compared to the effect of the rs1143634 SNP alone, suggesting that the SNP may be functional.

The second risk haplotype was composed of the *IL1B* rs1143633 minor allele and 3 major alleles (*IL1B* rs1143634, *IL1B* rs16944, and *IL1RN* VNTR allele 1). Our finding contradicts that reported by Moxley, *et al*[22], as the haplotype predisposing to hand OA in their study carried the *IL1RN* VNTR allele 2. In our sample, the 121-2 haplotype was rare (0.038 in subjects without bilateral DIP OA and 0.029 in subjects with bilateral DIP OA). Moreover, our results suggest that the observed effect is independent of the *IL1RN* VNTR polymorphism.

Earlier functional studies have suggested that the combination of alleles may be an important aspect in the regulation of the *IL1* gene expression. The *IL1RN* VNTR allele 2 has been associated with higher plasma levels of IL-1Ra than the *IL1RN* allele 1, but only in individuals who also had the T allele of *IL1B* (–511) polymorphism[14]. Vamvakopoulos, *et al*[16] observed that the *IL1RN* allele 2 was associated with higher IL-1Ra release, while subjects with 2 copies of the *IL1RN* allele 1 were found to release more IL-1ß than carriers of at least one *IL1RN* allele 2.

A balance between pro- and antiinflammatory cytokines regulates the inflammatory response. A decrease in the ratio of IL-1Ra to IL-1α and IL-1ß with increasing grades of knee OA has been detected[39]. An imbalance between pro- and antiinflammatory cytokines may contribute to the destructive process within the joint[42]. The risk haplotype identified in our study includes the alleles that affect the production of both IL-1Ra and IL-1ß. Thus we suggest that this haplotype is associated with a high production of IL-1ß and a low production of IL-1Ra and therefore leads to an alteration in the IL-1ß/IL-1Ra ratio.

Significance of occupation. Dentistry is one of the few occupations with an academic background that involves extensive bimanual work. Dentists perform arm movements repeatedly, often rapidly and for extended periods of time. Teachers represent an occupational group with a comparable academic background to the dentists, but with a distinctly different hand load. Repetitive movements with relatively low muscle activity may not result in muscle tissue damage, whereas continuous overload of finger joints resulting from highly monotonous usage may lead to joint impairment[48]. Previously we have shown that stereotyped repetitive tasks for prolonged periods of time increase the risk of OA in the joints of the thumb, index, and middle fingers among dentists[33]. Changes in the mechanical environment play an important role in modulating the production of proinflammatory mediators in articular cartilage. Injury of the joint may lead to considerable increases in local concentrations of proinflammatory cytokines[49]. The stronger associations between the *IL1* gene cluster polymorphisms and OA observed among the dentists, and interaction between the polymorphisms and variation in dental work tasks, produce additional support to the hypothesis that IL-1, and particularly IL-1ß, might be a key mediator in hand OA initiated by mechanical joint overload.

Study limitations and strengths. The relatively small number of subjects reduced the power of our study. The possibility that the observed association between the rs1143633 SNP and bilateral DIP OA might be spurious due to violation of HWE, among subjects without DIP OA, cannot be excluded. Departure from HWE, if not by chance, can be caused by multiple reasons such as population admixture, selection, sampling and genotyping error, or other biases[50]. The Finnish population is genetically relatively homogeneous and represents an isolated gene pool, the isolation being caused by historical, linguistic, and geographic factors. All study subjects were of Finnish origin. Genotyping was blinded towards DIP OA status. The genotype frequencies for all SNP in the study population (subjects with and without bilateral DIP OA combined) did not significantly deviate from the HWE. Genotyping error is also unlikely since the random sequencing of some of the patients was performed to verify the genotypes.

A strength of our study was the use of haplotypes; grouping of SNP in haplotypes leads generally to a stronger association with the phenotype than individual polymorphisms. We were also able to control for several potential confounders in statistical analyses.

Our results suggest an important role for the *IL1* gene cluster in the etiology of bilateral DIP OA and, further, that *IL1* polymorphisms may predispose DIP joints to the effects of mechanical overload. However, the possibility remains that the studied polymorphisms do not directly affect the individual susceptibility to hand OA, but are in LD with an unknown nearby susceptibility locus. The findings are to be weighed in future studies.

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