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. (J Rheumatol First Release August 15 2009; 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 population1. Risk factors of hand OA include age, sex, acute injury, repetitive joint loading, and obesity2.

Published data suggest that genetic factors play a major role in the etiology of OA3. Genetic susceptibility may be more relevant to OA in women than in men and may differ between joint groups4–5. Leppävuori, et al6 found evidence...
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-1β (IL1B on 2q13), IL-1 type I receptor (IL1R1 on 2q12), IL-1 type II 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-1β, and IL-1 receptor antagonist (IL-1Ra) are structurally related to one another and act by directly binding to IL-1 receptors. Both IL-1α and IL-1β are strong inducers of inflammation and recognize the same receptor, IL-1R. 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-1β and/or underproduction of IL-1Ra may predispose to local tissue destruction. IL-1 stimulates the synthesis and activity of matrix metalloproteinases and other enzymes involved in cartilage destruction in rheumatoid arthritis and OA.

All IL1 cluster genes are highly polymorphic. A single-nucleotide polymorphism (SNP) in the promoter region (C-889T; rs1800587) of the IL1A gene, SNP in the promoter region (C-511T; rs16944) and in exon 5 (C+3954T; rs1143634) of the IL1B gene, and a variable-number tandem repeat (VNTR) polymorphism in the intron 2 of the IL1RN gene 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.

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, while others have reported lack of association. 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. proposed that either excess or absence of mechanical force may induce the secretion of cytokines, such as IL-1β. Recent studies showed that moderate load inhibits the IL-1β, while its expression is upregulated with high loads.

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. 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-889T (rs1800587) genotypes were resolved by a polymerase chain reaction (PCR)-based method employing primers described by McDowell, et al. 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 IA1 (C) and the allele lacking the site as IA2 (T). The IL1B genotypes were determined using PCR-based methods essentially as described. Briefly, the IL1B C-511T (rs16944), C+3954T (rs1143634), and IL1B C+5810A (rs1143633) base changes
were identified by *AvaI*, *TaqI*, and *XcmI* restriction enzyme diges-
tions subsequent to PCR amplification, respectively.

The polymorphic region of the *ILIRN* gene containing a VNTR is
situated in the second intron of the gene. The *ILIRN* 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. 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 *ILIRI* (rs1465325, rs956730, rs2287047)
and *ILIRL2* (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 con-
taining 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 MgCl₂. PCR products were purified with SAP/ExoI treat-
ment 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 inde-
pendent readers interpreted the results. In every genotyping anal-
ysis, 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 *ILIRI*, *ILIRL2*, and *ILIRN* 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 activi-
ties (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 vari-
ation was empirically defined using cluster analysis with the K-
means algorithm. Three clusters were identified: cluster 1 (high
variation) consisted of 96 (33%) dentists who performed a combina-
tion of different tasks, cluster 2 (moderate variation) of 64 (22%)
dentists who spent about half their work time on restoration treat-
ment and endodontics and another half on prosthodontics, peri-
odontics 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/m², overweight or obese BMI ≥ 25.0 kg/m²). 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, addi-
tive, and recessive) for estimation of best fit for the risk of OA.
The SNPSpD method was used to calculate the p value threshold
for 5% significance.

The degree of pairwise linkage disequilibrium (LD) was calcu-
lated for each pair of SNP using Haploview software. Because the loci were in close proximity to each other, a haplotype analysis
was also performed to investigate whether the underlying LD con-
tributed to the nonindependence of these associations. The haplo-
types were statistically reconstructed from population genotype
data using the PHASE program with the Markov-chain method for
haplotype assignments. 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 com-
parisons 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 bilat-
eral 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 investi-
gated by stratification and by logistic regression analysis. To eval-
uate 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 varia-
tion 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 teach-
ers and 31% in dentists; p = 0.0001). Subjects with OA were
significantly older and had higher BMI than those without
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 rs1143636 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.

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

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

* 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 was found 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, separate haplotype analyses were conducted for the IL1R1-IL1RL2 and the IL1A-IL1B-IL1RN regions. The frequencies of haplotype alleles for the IL1R1-IL1RL2 and IL1A-IL1RN in subjects with and without bilateral DIP OA are presented in Table 3.

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 1.11–3.13, 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 1.92, 95% CI 1.11–3.13, p = 0.02; teachers: OR 1.35, 95% CI 0.80–2.26, p = 0.26).

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

<table>
<thead>
<tr>
<th>Carriage of Variant Allele</th>
<th>Level of Variation in Work Tasks</th>
<th>Counts (No/Yes)</th>
<th>OR (95% CI)†</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>High</td>
<td>40/6</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>Moderate</td>
<td>22/9</td>
<td>1.90 (0.55–6.55)</td>
<td>0.31</td>
</tr>
<tr>
<td>No</td>
<td>Low</td>
<td>48/24</td>
<td>3.13 (1.11–8.85)</td>
<td>0.03</td>
</tr>
<tr>
<td>Yes</td>
<td>High</td>
<td>33/17</td>
<td>4.44 (1.47–13.43)</td>
<td>0.008</td>
</tr>
<tr>
<td>Yes</td>
<td>Moderate</td>
<td>23/10</td>
<td>2.92 (0.87–9.73)</td>
<td>0.08</td>
</tr>
<tr>
<td>Yes</td>
<td>Low</td>
<td>33/26</td>
<td>4.84 (1.66–14.11)</td>
<td>0.004</td>
</tr>
</tbody>
</table>

† Adjusted for age, BMI, level of leisure time hand-loading physical activity, and smoking history. For abbreviations, see Table 1.
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 3. Extended haplotype frequency distribution according to bilateral DIP OA status.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Haplotype</th>
<th>Bilateral DIP OA</th>
<th>OR (95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No (n = 660–670)</td>
<td>Yes (n = 410–414)</td>
<td></td>
</tr>
<tr>
<td>IL1R1–</td>
<td>H1: 111</td>
<td>237</td>
<td>139</td>
<td>1.00</td>
</tr>
<tr>
<td>IL1RL2*</td>
<td>H2: 112</td>
<td>231</td>
<td>148</td>
<td>1.07 (0.79–1.45)</td>
</tr>
<tr>
<td></td>
<td>H3: 221</td>
<td>132</td>
<td>90</td>
<td>1.22 (0.85–1.74)</td>
</tr>
<tr>
<td></td>
<td>H4: 121</td>
<td>53</td>
<td>29</td>
<td>0.98 (0.57–1.86)</td>
</tr>
<tr>
<td></td>
<td>H5: rare</td>
<td>7</td>
<td>4</td>
<td>0.51 (0.08–3.04)</td>
</tr>
<tr>
<td>IL1B–IL1RN**</td>
<td>H1: 112–1</td>
<td>118</td>
<td>66</td>
<td>1.52 (1.01–2.28)</td>
</tr>
<tr>
<td></td>
<td>H2: 211–1</td>
<td>149</td>
<td>114</td>
<td>1.17 (0.76–1.79)</td>
</tr>
<tr>
<td></td>
<td>H3: 121–1</td>
<td>136</td>
<td>87</td>
<td>1.12 (0.73–1.74)</td>
</tr>
<tr>
<td></td>
<td>H4: 112–2</td>
<td>125</td>
<td>76</td>
<td>0.94 (0.55–1.60)</td>
</tr>
<tr>
<td></td>
<td>H5: 111–1</td>
<td>70</td>
<td>35</td>
<td>1.04 (0.60–1.79)</td>
</tr>
<tr>
<td></td>
<td>H6: rare</td>
<td>60</td>
<td>34</td>
<td></td>
</tr>
</tbody>
</table>

† 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.

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.

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**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).
Among patients with OA at multiple sites (hand, knee, hip, and spine) the innate inflammatory components are involved in the disease process. 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. IL-1α, IL-1β, and tumor necrosis factor-α have been detected in the synovial fluid and cartilage of patients with OA. Cartilage from an OA joint spontaneously produces a higher amount of IL-ß compared with normal cartilage. 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. The IL1B and IL1RN genes encode IL-1 receptors that have been shown to mediate the activation of nuclear factor-kB, which in turn participates in the regulation of inflammatory and immune-response gene expression.

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 rs114364 minor allele with increased risk of hip OA. The IL1B rs114364 minor allele was associated with increased risk of end-stage symptomatic knee or hip OA in 1 study and reduced risk of hip OA in another study. Stern and colleagues found an elevated risk of erosive hand OA in subjects homozygous for the IL1BA rs1143633 minor allele with another haplotype and is protective to another. Relatively strong LD in the IL1B-IL1RN extended haplotypes and occupation.

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 rs1143634 fitted a recessive mode and the IL1B rs1143634 a dominant mode of inheritance. In addition, we observed a statistically significant interaction between both the IL1RI-IL1RL2 and IL1B-IL1RN extended haplotypes and occupation.

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Significance of haplotype. Relatively strong LD in the IL1 gene cluster has been reported. 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.

Table 4. The IL1RI-IL1RL2 and IL1B-IL1RN extended haplotype frequency distribution according to bilateral DIP OA status by occupation.

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Dentists No Bilateral DIP OA Frequency</th>
<th>Dentists Bilateral DIP OA Frequency</th>
<th>Teachers No Bilateral DIP OA Frequency</th>
<th>Teachers Bilateral DIP OA Frequency</th>
<th>OR (95% CI)</th>
<th>p</th>
<th>OR (95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>H1: 111</td>
<td>149 0.38 51 0.28 1.00</td>
<td>86 0.33 90 0.39 1.00</td>
<td>0.144***</td>
<td></td>
<td></td>
<td></td>
<td>0.336***</td>
<td></td>
</tr>
<tr>
<td>H2: 112</td>
<td>130 0.33 78 0.42 1.75 (1.15–2.68)</td>
<td>104 0.40 72 0.32 0.66 (0.43–1.01)</td>
<td>0.119</td>
<td></td>
<td></td>
<td></td>
<td>0.163***</td>
<td></td>
</tr>
<tr>
<td>H3: 221</td>
<td>82 0.21 41 0.22 1.48 (0.90–2.42)</td>
<td>49 0.19 49 0.22 0.96 (0.58–1.59)</td>
<td>0.09</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H4: 121</td>
<td>30 0.07 14 0.08 1.36 (0.67–2.77)</td>
<td>21 0.08 15 0.07 0.68 (0.33–1.41)</td>
<td>0.392</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H5: 112–1</td>
<td>7 0.01 0 0.00 —</td>
<td>2 0.01 2 0.01 0.96 (0.13–6.94)</td>
<td>0.964</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H6: 11–2</td>
<td>7 0.01 0 0.00 —</td>
<td>2 0.01 2 0.01 0.96 (0.13–6.94)</td>
<td>0.964</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

† Adjusted for age, BMI, and smoking history. For IL1RI, IL1RL2, and IL1B polymorphisms 1 = common allele, 2 = rare allele. For the IL1RNA VNTR allele 1 has 4 repeat units; allele 2 has 2 repeat units. * Allele positions were ordered as follows: rs1143633, rs1143634, rs1143634, rs16944, and IL1RN VNTR. p for global association. For abbreviations, see Table 1.
(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 IL1A-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-1ß 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.

REFERENCES


14. Hurme M, Santtila S. IL-1 receptor antagonist (IL-1ra) plasma levels are co-ordinately regulated by both IL-1Ra and IL-1-beta genes. Eur J Immunol 1998;28:2598-602.


