Research article

Vitamin D receptor gene polymorphisms and susceptibility of hand osteoarthritis in Finnish women

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Abstract

We examined whether polymorphisms of the vitamin D receptor (VDR) gene was associated with individual risk of hand osteoarthritis (OA). Radiographs of both hands of 295 dentists and of 248 teachers were examined and classified for the presence of OA using reference images. The VDR Apa and TaqI genotypes were determined by PCR-based methods. No association was observed between the VDR polymorphisms and the odds of overall hand OA. However, the carriers of the VDR t allele or At haplotype were at almost half the odds of symmetrical hand OA (OR = 0.60, 95% CI = 0.38–0.94 and OR = 0.59, 95% CI = 0.38–0.93, respectively) compared with the carriers of the T allele and of the non-At haplotype, respectively. Increased odds of this disease, on the contrary, was observed for women with two copies of the VDR a allele (OR = 1.93, 95% CI = 1.99–3.70) compared with women with the AA genotype. Conversely, the VDR a allele carriage was associated with a tendency of lowered odds of osteophyte (OR = 0.51, 95% CI = 0.25–1.03). When the genotype data were used to construct haplotypes, the VDR AaTt joint genotype appeared to pose a remarkably lower odds (OR = 0.26, 95% CI = 0.08–0.91) of osteophyte compared with the AAtt joint genotype. As a novel finding we observed a joint effect of a low calcium intake and VDR polymorphisms on symmetrical OA; the OR was 2.64 (95% CI = 1.29–5.40) for carriers of the aT haplotype with low daily calcium intake compared with non-carriers of the haplotype with high daily calcium intake. Our results suggest that VDR gene polymorphisms play a role in the etiology of symmetrical hand OA. Moreover, the association between the VDR gene and OA may be modified by calcium intake.

Introduction

Osteoarthritis (OA) is the most frequent cause of musculoskeletal disability in developed countries. Two main subsets of the disease are recognized: monoarticular OA and polyarticular OA [1]. Despite the fact that OA is the most common joint disease, its etiology remains unclear. Among the most commonly suspected risk factors are age [2], previous injury [3], and obesity [2,4]. Current evidence suggests a genetic component to OA, with the heritability for hand OA and knee OA ranging from 39% to 65% [5,6].

Some of the studies have proposed that genetic susceptibility may be more relevant to OA in women than to OA in men, and that the role of genes in the development and progression of OA may vary between joint groups [7].

Hand OA often aggregates with knee OA [5,8,9]. Epidemiologic studies have shown that women with radiographic changes in the knee or the hand have an increased bone mass after adjustment for age and other covariates as compared with those women without OA [10,11]. Genetic factors affecting bone density may therefore play a role in the development of OA.

The vitamin D endocrine system, consisting of the steroid vitamin D, the vitamin D receptor (VDR) and the metabolizing

BMI = body mass index; CI = confidence interval; DIP = distal interphalangeal; MCP = metacarpophalangeal; PCR = polymerase chain reaction; PIP = proximal interphalangeal; OA = osteoarthritis; OR = odds ratio; VDR = vitamin D receptor.
enzymes, plays an important role in skeletal metabolism, OA, the immune response, and cancer [12]. The VDR gene acts as an important regulator of calcium metabolism and bone cell function [13], and it was the first candidate gene to be studied in osteoporosis. Biological support for an association between the VDR genotype and OA comes from studies showing that serum levels of vitamin D are related to the progression of knee OA [14] and to incident changes of radiographic hip OA characterized by joint space narrowing [15].

It has been shown that common allelic variations in the VDR locus can be used to predict the bone turnover rate [16]. The polymorphisms at the 3' end of the gene include two polymorphisms detectable with BsmI and Apal restriction enzymes that are located in intron 8 and one polymorphism detectable with TaqI restriction enzyme located in exon 9, while a poly(A) microsatellite polymorphism is located in the 3' untranslated region of the gene. The Apal, BsmI, and TaqI polymorphisms have been shown to be in strong linkage disequilibrium [16].

Keen and colleagues [17] examined the VDR TaqI polymorphism, associated with bone mineral density and osteoporosis, in relation to OA among postmenopausal English women. They found that women carrying one or two TaqI wild-type alleles (T) had an approximately threefold risk of OA in the knee joints compared with homozygotes for the variant allele (t). Uitterlinden and colleagues [18,19], on the other hand, demonstrated that a VDR aT haplotype (the T allele together with variant a allele at the Apal locus) was related to knee OA, especially in osteophyte formation among elderly Dutch men and women.

It can be expected that the same polymorphisms could be important for both knee OA and hand OA, although no association between the VDR genotypes and OA was found in Japanese women [20] and in American men and women [21]. Various factors may have contributed to these apparently discordant results among studies, including the differences in the VDR genotypes distribution, the genetic environment and age structure of the studied population [22], and, more specifically, failure to take into account factors that modulate the effect of VDR gene on the risk of OA. Observations that the VDR alleles are located in intron 8 and one polymorphism detectable with TaqI restriction enzyme located in exon 9, while a poly(A) microsatellite polymorphism is located in the 3' untranslated region of the gene. The Apal, BsmI, and TaqI polymorphisms have been shown to be in strong linkage disequilibrium [16].

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The Finnish population is genetically relatively homogeneous and represents an isolated gene pool, the isolation being caused by linguistic and geographic factors. To our knowledge, the association between the VDR gene polymorphisms and OA has not been studied in the Finnish population. The aim of the present study was to examine whether the VDR polymorphisms, which were previously associated with knee OA, are also associated with the risk of hand OA in Finnish middle-aged women. In addition, we evaluated whether the calcium intake modifies the relationship between the VDR gene polymorphisms and hand OA.

**Materials and methods**

**Subjects**

The potential study subjects were identified through the register of the Finnish Dental Association and the Finnish Teachers Trade Union. Four hundred and thirty six women aged 45–63 years were randomly selected from each occupational group using the place of residence as 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 between October 2002 and March 2003. 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.

**Hand radiography and image analysis**

Both hands of the participants were radiographed. Kodak X-ray films were exposed with Siemens X-ray equipment (48 kV, 10 mA, focus film distance = 115 cm; Siemens, Munich, Germany). The analogue radiographs were evaluated by an experienced radiologist (TV) who was blinded to the occupation, age, and all health data of the subjects.

Each distal interphalangeal (DIP) joint, proximal interphalangeal (PIP) joint, thumb interphalangeal joint, and metacarpophalangeal (MCP) joint of both hands was graded separately, and was classified for the presence of OA using a modified Kellgren and Lawrence system [27]. The classification criteria were: grade 0 = no OA, grade 1 = doubtful OA, grade 2 = mild OA, grade 3 = moderate OA, and grade 4 = severe OA [22]. Osteophytes were separately classified as absent (grade 0), minimal or questionable (grade 1), or distinct (grade 2). Reference images were used. The description of reference images has been given elsewhere [28].

The reliability of the readings was estimated by measuring intraobserver and interobserver agreements, using the weighted Cohen's kappa coefficient with quadratic weights [29], for 46 randomly chosen subjects. For this estimation, a second reading was independently performed by TV and another experienced radiologist (KL). The inter-observer agreement for OA ranged from 0.67 to 0.85 for DIP joints, from 0.39 to 0.61 for PIP joints, and from 0.18 to 0.69 for MCP joints. The intraobserver agreement for OA ranged from 0.73 to 0.88 for DIP joints, from 0.67 to 0.92 for PIP joints, and from 0.59 to 1.0 for MCP joints. The readings of TV only were used in the subsequent statistical analyses.
If the subject had at least three finger joints with radiographic OA of grade 2–4, she was classified as having finger OA. Symmetrical OA was defined as a subcategory of OA in at least two symmetrical pairs of the joints (if two joints of the hand are affected, the same joints of the opposite hand are also affected). If the subject had at least two finger joints with distinct osteophyte (grade 2), she was classified as having finger osteophyte.

**Genotyping analysis**
All DNA samples were extracted from lymphocytes by a DNA extraction kit (PUREGENE® DNA Purification Kit; Gentra Systems, Plymouth, MN, USA).

The VDR Apal and TaqI genotypes were resolved by a PCR-based method employing primers described by Riggs and colleagues [30]. Briefly, the PCR reactions were set up as follows: 50–100 ng template, 1 U DNA polymerase (Biotools; B&M Labs, SA, Madrid, Spain), 0.2 mM dNTPs, 0.5 μM each primer and 1.5 mM MgCl₂ in the magnesium-free PCR buffer (Biotools; B&M Labs, SA). After initial denaturation of 2 min at 94°C, 28 cycles of 10 s at 94°C, 20 s at 60°C, and 30 s at 72°C were performed, followed by a 5 min final extension at 72°C. Aliquots of the PCR products were digested with Bsp120I (Fermentas) and TaqI (Fermentas) for Apal and TaqI polymorphisms, respectively, and were electrophoresed on 3% agarose gel containing ethidium bromide. The final results were interpreted from pictures of the gels photographed under UV light; alleles lacking restriction sites for Apal and TaqI were denoted as VDR A and T alleles, and alleles with the restriction sites as a and t, respectively.

**Questionnaires**
Data regarding individual characteristics were collected by a self-administered questionnaire that included items on anthropometric measures, use of hormone therapy, dietary habits, and smoking history.

The use of hormone therapy was assessed by the questions ‘Do you use hormonal medication? If yes, what is the name of medication?’ and ‘How long have you been using this hormonal medication?’

Queries about dietary habits on a daily average basis included coffee consumption (the number of cups), milk/sour milk consumption (the number of cups, one cup = 2 dl), yogurt consumption (the number of deciliters), and cheese consumption (the number of slices). Alcohol consumption was reported on a weekly average basis (number of portions, one portion = 12 cl for wine, 0.33 l for beer, and 4 cl for vodka or liquor).

Daily calcium intakes from particular dairy products were calculated from a knowledge of amounts of calcium per 100 g (1 dl) of each dairy product and amount of each product consumed per day. Subjects were asked about their daily intake of calcium from vitamin supplements. Daily calcium intake was determined by adding up the calcium level of the dairy products and the amount of calcium from vitamin supplements. For the analysis, daily calcium intake was compared with Finnish limit values [31] and was classified into low intake (<400 mg/day), adequate intake (400–800 mg/day), and high intake (>800 mg/day).

**Statistical analyses**
Allele and genotype frequencies were compared between individuals with and without OA using Fisher’s exact probability test or the chi-square test. Carriage rates for the alleles were calculated as the proportion of individuals with at least one copy of the allele. Each gene locus was also examined for an allele dosage effect, by comparing the numbers of individuals heterozygous and homozygous for the test allele among those with and without OA.

The VDR haplotypes were statistically reconstructed from population genotype data using the PHASE program with the Markov chain method for haplotype assignments [32].

Crude and adjusted ORs and their 95% confidence intervals (CIs) were calculated using the SPSS statistical package (SPSS Inc., Chicago, IL, USA). The ORs were adjusted for the potential confounding factors of age, occupation, height, BMI, use of hormone therapy, daily calcium intake, and coffee and alcohol consumption. Since the crude and adjusted ORs did not differ significantly, only the adjusted ORs are shown.

**Results**
The prevalence of the overall hand OA, symmetrical OA, and osteoarthropathies among the female dentists and teachers aged 45–63 years were 29%, 21%, and 7%, respectively. The
smokers was higher among the carriers of the VDR **aa** polymorphisms and the overall odds of hand OA. However, the VDR **Aa** genotype posed an almost doubled odds of symmetrical OA (OR = 1.93, 95% CI = 1.00–3.70) compared with the **AA** genotype (Table 2). The women with at least one copy of the VDR **t** allele (Table 2) or carrying the **At** haplotype (Table 3) were at almost halved odds of this disease (OR = 0.60, 95% CI = 0.38–0.94 and OR = 0.59, 95% CI = 0.38–0.93, respectively) compared with the carriers of the **T** allele and the non-**At** haplotype, respectively. On the other hand, the VDR **a** allele carriage was associated with a tendency (OR = 0.51, 95% CI = 0.25–1.03) to lowered odds of osteophyte. The VDR **AaTt** joint genotype appeared to pose a remarkably lower odds of osteophyte (OR = 0.26, 95% CI = 0.08–0.91) compared with the **AAtt** joint genotype (Table 3).

The separate and joint effects of the VDR **aT** haplotype and a low calcium intake on the risk of hand OA are presented in Table 4. The presence of the **aT** haplotype alone was associated with an increased odds of symmetrical OA (OR = 1.58, 95% CI = 0.89–2.85) and with a decreased odds of osteophytes (OR = 0.39, 95% CI = 0.18–0.85). Similarly, there was no statistically significant association between a low calcium intake and OA among subjects without the **aT** haplotype. However, both factors (the **aT** haplotype and low calcium intake) acted synergistically to increase the odds of OA. We observed a joint effect of a low calcium intake and carriage of the VDR **aT** haplotype on symmetrical OA; the OR was 2.64 (95% CI = 1.29–5.40) for carriers of the **aT** haplotype with a low daily calcium intake compared with non-carriers of the haplotype with a moderate or high daily intake.

In agreement with previous observations [27] that the VDR **a** allele appeared to always be associated with the **T** allele, identical ORs for the VDR **a** allele carriage and the **aT** haplotype carriage were seen in the present study (Tables 2 and 3).

### Discussion

An age-dependent pattern for the presence of finger OA has been found among adult participants of the Tecumseh Community Health Study [34]. Among individuals under the age of
Table 2

Association between the VDR genotypes and hand osteoarthritis (OA)

| Genotype | Overall OA Yes/no | Odds ratio (95% CI)a | Symmetrical OA Yes/no | Odds ratio (95% CI)a | Osteophyte Yes/no | Odds ratio (95% CI)a |
|----------|------------------|----------------------|-----------------------|----------------------|------------------|----------------------|
| Apalb    |                  |                      |                       |                      |                  |                      |
| AA       | 51/123           | 1.0                  | 29/145                | 0.98 (0.62–1.54)     | 14/254           | 0.46 (0.21–1.00)     |
| Aa       | 77/191           | 1.0                  | 56/212                | 1.43 (0.84–2.42)     | 14/254           | 0.46 (0.21–1.00)     |
| aa       | 32/69            | 1.0                  | 25/76                 | 1.93 (1.00–3.70)     | 7/94             | 0.65 (0.24–1.75)     |
| 'A' allele carriage | 128/303 | 1.0 | 85/357 | 1.55 (0.94–2.56) | 21/348 | 0.51 (0.25–1.03) |
| 'a' allele carriage | 109/260 | 1.03 (0.67–1.59) | 81/288 | 1.55 (0.94–2.56) | 21/348 | 0.51 (0.25–1.03) |
| Taqc    |                  |                      |                       |                      |                  |                      |
| tt      | 20/44            | 1.0                  | 11/53                 | 1.0                  | 7/57             | 1.0                  |
| Tt      | 68/173           | 0.75 (0.39–1.44)    | 42/199                | 0.90 (0.41–1.97)     | 13/228           | 0.46 (0.16–1.26)     |
| TT      | 71/164           | 1.0                  | 57/178                | 1.53 (0.72–3.29)     | 19/216           | 0.73 (0.27–1.93)     |
| 't' allele carriage | 88/217 | 0.87 (0.58–1.30) | 53/252 | 0.60 (0.38–0.94) | 20/285 | 0.77 (0.39–1.53) |
| 'T' allele carriage | 139/337 | 0.83 (0.45–1.54) | 99/377 | 1.19 (0.57–2.47) | 32/444 | 0.58 (0.23–1.46) |

aOdds ratio and 95% confidence interval (CI) adjusted for age (years), height (cm), occupation (1 = dentists, 2 = teachers [reference group]), hormone therapy (1 = ever used, 2 = never used [reference group]), daily calcium intake (1 = high [reference group], 2 = moderate, 3 = low [reference group]), coffee consumption (number of cups per day), alcohol consumption (number of portion per week), and smoking history (1 = never smoked [reference group], 2 = ever smoked). bA, absence of a restriction site; a, presence of a restriction site. cT, absence of a restriction site; t, presence of a restriction site. For technical reasons, three samples were not genotyped for the TaqI VDR polymorphism.

Table 3

Association between the VDR haplotypes and hand osteoarthritis (OA)

| Joint genotypea | Overall OA Yes/no | Odds ratio (95% CI)b | Symmetrical OA Yes/no | Odds ratio (95% CI)b | Osteophyte Yes/no | Odds ratio (95% CI)b |
|-----------------|------------------|----------------------|-----------------------|----------------------|------------------|----------------------|
| Aatt            | 20/44            | 1.0                  | 11/53                 | 1.0                  | 7/57             | 1.0                  |
| AATt            | 26/64            | 0.75 (0.35–1.61)    | 14/75                 | 0.73 (0.29–1.83)     | 8/82             | 0.82 (0.2–2.56)      |
| AATT            | 5/15             | 0.68 (0.20–2.38)    | 4/16                  | 1.03 (0.26–4.08)     | 3/17             | 1.62 (0.33–7.92)     |
| AaTt            | 43/111           | 0.75 (0.38–1.50)    | 28/125                | 0.99 (0.43–2.25)     | 5/149            | 0.26 (0.08–0.91)     |
| AaTT            | 34/80            | 0.89 (0.43–1.84)    | 28/86                 | 1.55 (0.67–3.54)     | 9/105            | 0.70 (0.23–2.13)     |
| AaTT            | 32/69            | 1.00 (0.48–2.07)    | 25/76                 | 1.63 (0.70–3.82)     | 7/94             | 0.61 (0.19–2.00)     |
| At haplotype carriage | 89/219 | 0.87 (0.58–1.30) | 53/253 | 0.59 (0.38–0.93) | 20/288 | 0.76 (0.38–1.52) |
| AT haplotype carriage | 65/159 | 0.93 (0.62–1.41) | 46/177 | 0.96 (0.61–1.52) | 20/204 | 1.60 (0.78–3.27) |
| aT haplotype carriage | 109/260 | 1.03 (0.67–1.59) | 81/287 | 1.55 (0.94–2.56) | 21/348 | 0.51 (0.25–1.03) |
| No aT haplotypec | 51/123           | 1.0                  | 29/144                | 1.0                  | 18/156           | 1.0                  |
| One aT haplotyped | 77/191           | 0.98 (0.62–1.54)    | 56/213                | 1.43 (0.84–2.42)     | 14/254           | 0.46 (0.21–1.00)     |
| Two aT haplotypesg | 32/69            | 1.20 (0.67–2.15)    | 25/76                 | 1.93 (1.01–3.70)     | 7/94             | 0.65 (0.24–1.75)     |

aA, absence of a restriction site; a, presence of a restriction site; T, absence of a restriction site; t, presence of a restriction site. bOdds ratio and 95% confidence interval (CI) adjusted for age (years), height (cm), occupation (1 = dentists, 2 = teachers [reference group]), hormone therapy (1 = ever used, 2 = never used [reference group]), current body mass index (1 = high, 2 = moderate, 3 = low [reference group]), daily calcium intake (1 = high [reference group], 2 = moderate, 3 = low), coffee consumption (number of cups per day), alcohol consumption (number of portion per week), and smoking history (1 = never smoked [reference group], 2 = ever smoked). cNo aT haplotype = AATT + AaTt + AaTT. dOne copy of the aT haplotype = AaTT + AaTt. gTwo copies of the aT haplotype = aaTT.
44 years OA was observed almost exclusively in the DIP joints, whereas among older participants the PIP and MCP joints were affected. Several studies provided clear evidence for a polyarticular subset of hand OA in women [28,35-37], with three major determinants of the pattern of polyarticular involvement being symmetry, clustering by row, and clustering by ray (in descending order of importance). While OA in a specific joint (monoa rticular OA) may result from acute trauma [27] or from mechanical stress [38] to the joint, OA with multiple joint involvement (polyarticular OA) might be dominated by systemic factors to which all joints would be equally susceptible.

In the present study we aimed to examine more severe cases of OA, which are more likely to bear a genetic component. OA was therefore defined to be present if there was a radiograph reading of grade 2–4 in at least three joints of the fingers. OA was defined to be symmetrical if at least two symmetrical pairs of joints (the same joint in both hands) were affected.

Our findings suggest that there may indeed be a relationship between the VDR Apal and TaqI polymorphisms and the risk of symmetrical hand OA in Finnish women. The VDR aa genotype, which has previously been associated with high bone mass [30], posed a nearly twofold increased odds of symmetrical hand OA as compared with the AA genotype associated with lower bone mass. In contrast, the odds of this disease was almost halved among those with the VDR t allele, which has previously been associated with a higher rate of bone turnover [39] and lower bone mass [40] compared with the T allele.

In our study, the association between the VDR aT haplotype and hand OA depended on the interaction with dietary calcium intake. A joint effect of a low calcium intake and carriage of the VDR aT haplotype on risk of symmetrical OA exceeded their additive effects, indicating that the VDR aT haplotype is a potential risk factor for OA among women with insufficient calcium intake. These relationships were independent of other risk factors such as age, occupation, BMI, use of hormone therapy, and smoking history.

Regulation of intracellular calcium plays a key role in hypertension, insulin resistance, and obesity [41]. A protective effect of dietary calcium in preventing bone fragility and certain cancers has been reported [42]. Several previous studies have recognized that the association between VDR alleles and bone mineral density may vary depending on the level of calcium intake [25,26,43-45], and there is evidence that calcium is able to enhance cartilage repair and stimulate collagen production [46]. The VDR baT haplotype has been related to enhanced abnormality in the calcium regulation of the parathyroid hormone secretion from adenomatous parathyroid cells of primary human parathyroid tumor [47]. However, no other study has evaluated the potential relation between calcium intake and VDR genotypes in OA etiology. Our finding, if confirmed, implies a considerable potential for a role of nutritional interventions for OA.

In the Framingham study [14] the growth of knee osteophytes was found to be associated with the vitamin D level. In the present study, women with the VDR AaTt joint genotype had the lowest odds and those with the AATT genotype the highest odds of hand osteophytes as compared with the AA genotype. The direction of these associations contrast the findings in the studies of osteophytes formation in the knee [17-19] and the lumbar spine [48], but agree with the findings related to the severity and presence of lumbar spine osteophytes [49].

The large amount of positive genetic association data in a number of diseases such as osteoporosis, OA, diabetes, and cancer [50] suggest functional consequences of VDR gene polymorphisms. The Apal polymorphism is unlikely to have functional consequences, however, since it is located in intron 8 and is not affecting any splicing site or transcription factor binding site. Moreover, although the TaqI polymorphism is located in the coding sequence (exon 9) of the VDR gene, it has no effect to the encoded protein sequence [13]. Nevertheless, supporting the modifying role of the VDR polymorphisms in the VDR functionality, lower VDR mRNA levels were found to be associated with homozygosity for the a and T alleles [51].

### Table 4

| Carriage of the aT haplotype | Low calcium intake | n | Overall OA | Symmetrical OA | Osteophyte |
|-----------------------------|-------------------|---|------------|----------------|------------|
| No                          | No                | 131| 1.0        | 1.0            | 1.0        |
| No                          | Yes               | 43 | 1.02 (0.46–2.30) | 1.73 (0.69–4.36) | 0.28 (0.06–1.35) |
| Yes                         | No                | 269| 0.92 (0.56–1.51) | 1.56 (0.88–2.85) | 0.39 (0.18–0.85) |
| Yes                         | Yes               | 100| 1.58 (0.85–2.93) | 2.64 (1.29–5.40) | 0.52 (0.17–1.56) |

Data presented as the odds ratio (95% confidence interval) adjusted for age (years), height (cm), occupation (1 = dentists, 2 = teachers [reference group]), current body mass index (1 = high, 2 = moderate, 3 = low [reference group]), coffee consumption (number of cups per day), alcohol consumption (number of portion per week), and smoking history (1 = never smoked [reference group], 2 = ever smoked).
Our study has several limitations. The cross-sectional study design precluded the assessment of an effect of dietary calcium intake on the incidence or progression of OA. The relatively small number of subjects reduced the power of the study to detect differences. Another limitation arises from the fact that calcium intake was assessed based on a questionnaire, and thus the recall bias may affect the accuracy of information gathered. Finally, vitamin D intake was not assessed in our population.

Despite these limitations, the present findings are not anticipated to be caused by selection bias. First, selection on the genotype is unlikely since all study subjects were of homogeneous Finnish origin. Second, the prevalence of hand OA among the women analyzed in this study is similar to that seen in other studies [52-54]. Third, the VDR genotype frequencies in this population did not significantly deviate from the Hardy-Weinberg equilibrium and the genotype frequencies were similar to those previously observed in Finnish women [33]. Neither could the associations be explained by other risk factors, since these potential confounders were controlled in all statistical analyses.

The lower level of interobserver agreement for radiographic findings was not surprising. Despite training and the use of reference images, each reader graded the radiographs according to his or her own inherent standard about what constituted a positive finding. The high intraobserver agreement suggests that the classification criteria applied here are highly reproducible. Because all radiographs were evaluated by one observer who was blind to subjects’ genetic data, the high intraobserver repeatability implies that the comparison between subjects with different VDR genotypes was unbiased. The possibility of non-differential misclassification of osteoarthritis cannot be ruled out. Non-differential misclassification of a binary outcome usually produces bias toward the null.

It could be argued that at least some of the associations found in this study were spurious, considering multiple comparisons were performed. However, there are several arguments to support the consistency of these results rather than attributing them to chance. First, we hypothesized a priori that the interaction observed in this study would occur, based on the known biology of the VDR gene in regulation of calcium metabolism. Second, our sample was homogeneous in terms of age, gender, ethnicity, and genetic background. Third, haplotypes were constructed taking into account the knowledge that collective grouping of single nucleotide polymorphisms in haplotypes has a stronger association with the phenotype than individual polymorphisms. Finally, to minimize the number of analyses performed, the interaction hypothesis was tested with the risk haplotype only.

Conclusion
Although the possibility remains that the studied polymorphisms do not directly affect the individual susceptibility of hand OA, but are in linkage disequilibrium with an unknown nearby susceptibility locus, our results provide evidence of the involvement of the VDR polymorphism in the etiology of symmetrical hand OA. In addition, our findings suggest a detrimental effect of low dietary calcium intake on OA. The findings remain to be weighted in future studies. Further studies into mechanisms underlying the relationships between calcium and OA may improve the understanding of the obtained results.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
SS participated in the design of the study, performed the statistical analysis, and drafted the manuscript. AH and PS carried out the genotyping analysis. TV carried out the evaluation of hand radiographs. KL participated in the evaluation of reliability of radiographs’ readings. HR conceived of the study and participated in its design. PLA conceived of the study and participated in its design and coordination. All authors read and approved the final manuscript.

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