Absence of Linkage or Association for Osteoarthritis with the Vitamin D Receptor/Type II Collagen Locus: The Framingham Osteoarthritis Study

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ABSTRACT. ***Objective***. Studies have suggested that polymorphisms or mutations either in the *COL2A1* or *VDR* gene, both on chromosome 12q, are associated with the occurrence of osteoarthritis (OA).We examined linkage and association between the VDR/COL2A1 locus and hand/knee OA in the Framingham Osteoarthritis Study (FOS).

Methods. Hand and knee joints were characterized radiographically in the FOS. An overall score for OA using the standard Kellgren and Lawrence grading scheme was determined, as well as scores for individual features of OA including osteophytes and joint space narrowing. For linkage studies, polymorphic microsatellite markers near the *VDR-COL2A1* genes on chromosome 12 were tested in a collection of 296 of the largest Framingham Heart Study families and the results analyzed using variance component linkage (SOLAR). For association studies, we characterized the allele status of a subset of subjects at the BsmI site of the *VDR* gene.

Results. Overall, we found no linkage or association between OA and the COL2A1/VDR locus for either knee or hand OA, nor did we find an association or linkage between *COL2A1* or *VDR* with any individual radiographic features of OA.

Conclusion. Despite studies suggesting associations of OA with both COL2A1 and VDR loci, our results suggest that mutations at the COL2A1/VDR locus do not play an important role as a cause of common OA in the population at large. (J Rheumatol 2002;29:161–5)

Key Indexing Terms:
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Osteoarthritis (OA) is a heterogeneous and multifactorial disease characterized by progressive degeneration of articular cartilage and by joint pain, discomfort, and immobility and is the most common form of arthritis in the United States and throughout the world[1-3]. While OA is common in the knee, it is even more prevalent in the hands.

Several pathologic mechanisms have been implicated in the development of OA including obesity, joint injury, metabolic diseases, bone and joint malformations, and genetic factors[4-7]. Several chromosomal locations have been identified that contribute to common OA[8-10]; however, the specific genes have yet to be identified. In a limited number of families, OA appears to be transmitted in a clear Mendelian dominant fashion suggesting the presence of a single genetic defect causing disease[11-16]. However, the disease in these families is atypical of what is seen in common OA[17].

The vitamin D receptor gene (*VDR)* is located on human chromosome 12q, and polymorphisms in the *VDR* or *COL2A1* gene have been associated with OA in some[18-22] but not all studies[23-25]. One positive study reported an association of *VDR* polymorphisms with osteophyte development[18] in contrast to other radiographic features of disease. Vitamin D plays a major role in bone mineral homeostasis by promoting the transport of calcium and phosphate to ensure that the blood levels of these ions are sufficient for normal bone mineralization. *COL2A1*, the gene encoding type II collagen, is another candidate gene for OA and is located in close proximity to the *VDR* gene. *COL2A1* has been associated with several rare cartilage disorders, such as chondrodysplasia, that frequently result in OA[12,15-17].

To understand the potential role of *VDR* and *COL2A1* in common OA, we evaluated linkage between OA and the

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VDR/COL2A1 locus in the Framingham Osteoarthritis Study (FOS), a sub-study within the Framingham Heart Study (FHS). In addition, we tested allelic association for the BsmI restriction polymorphism in the *VDR* gene to OA. Our study is unique in that both the parents and offspring were radiographically examined at similar adult ages when OA is prevalent. The results suggest that the *VDR/COL2A1* genes do not play a major role in common forms of OA.

MATERIALS AND METHODS

The Framingham Heart Study is a population based, multigenerational cohort study that began in 1948 and includes over 1000 extended kinships[26]. The original cohort (parents in the families studied here) were evaluated 3 times for the occurrence of OA[27] in either the hands or knees (1967–70 PA hand radiographs; 1983–85 AP weight bearing knee radiographs; 1992–93 both hand and knee radiographs). For the purpose of this study, the first radiograph of hands and knees in a subject was used. As part of a study investigating the heritability of OA (1993–94), we evaluated the Framingham offspring cohort who had at least one parent included in the previous OA studies using the same techniques. OA was characterized radiographically using the standard Kellgren and Lawrence (K&L) grading scheme[28]. To ensure that radiographs in offspring and cohort subjects would be read by the same standards, one reader read all hand radiographs with films from offspring and cohort mixed. A similar procedure was used to evaluate knee radiographs (see Reference 27 for radiographic reading methods and reproducibility). Hand and knee radiographs were also read for osteophytes, with each one scored based on size on a 0–3 scale and for joint space narrowing (0–3 scale) using the Framingham OA atlas[29].

Hand OA was defined as a quantitative trait as described[27]. Information from the distal interphalangeal (DIP) joints, proximal interphalangeal (PIP) joints, metacarpophalangeal (MCP) joints, and carpometacarpal joint at the base of each thumb was used, as these are the joints in the hand most commonly affected by OA. The K&L score at each joint was summed, across 10 DIP, 8 PIP, 10 MCP, and 2 thumb base joints. Multiple linear regression models for each sex were used to adjust for age, age squared, and age cubed. Using the standardized residuals from the regression models, we calculated normalized deviates on the ranks of the residuals to ensure normality of these measures. The normalized deviates have means of 0 and a standard deviation of 1, so that a deviate above 0 signifies more than average sum of scores with radiographic OA, and below 0, less than average. We also performed the above calculations for the proportion of hand joints with OA, defined as the proportion of joints with a K&L grade of 2 or more. Normalized ranks were computed for the sums of the osteophyte scores and the joint space narrowing scores as described for the K&L grades. The first hand radiograph of cohort members was of the right hand only, thus OA scores for these readings were doubled as described[27].

For knee OA, the standardized residuals were calculated for each knee using the K&L grade, the sums of osteophytes (sum over 4 sites), and the joint space narrowing measurements (sum over 2 sites) after adjusting for age, body mass index, and history of knee injury or operation. The normal scores were then computed based on the ranks of the sums of the standardized residuals of the left and the right knees.

DNA from the largest 330 families was sent to the Mammalian Genotyping Service (Marshfield, WI, USA). Of these families, 296 extended pedigrees had information on the OA traits derived from about 1470–1475 subjects with hand measurements and 1200 with knee measurements (Table 1). These individuals were evaluated for linkage in this study. The microsatellite markers D12S1090 (56 cM from p-ter), GATA91H06 (56 cM from p-ter), and D12S398 (68 cM from p-ter) flanking the region containing the *VDR-COL2A1* gene were tested. The *COL2A1* gene is located about 50,000 kb (65 cM) from the p-ter and is located in genomic segment AC004801 (Genbank, NCBI database) and the *VDR* gene located about 200 kb distal to COL2A1 in segment AC004466 (Genbank). All the markers tested had heterozygosity values between 0.67 and 0.87. The *VDR* polymorphism was assayed as described[30].

The genotyping data were analyzed by the following methods. For linkage analyses we employed the variance component model implemented in SOLAR[31] to normalized deviates derived from the standardized residuals so that these measures were normally distributed. In this model, linkage is represented by a variance component based on the identity by descent values at a specific marker. If the trait varies with the magnitude of identity by descent, linkage is found at that marker. Likelihood ratio tests are employed to compare a model with this variance component to a general polygenic model. To calculate multipoint linkage values, SOLAR uses the Fulker-Cherney regression approach[32].

Individuals were randomly selected for the association study as described[30]. Briefly, subjects who had bone mineral density (BMD) measure and DNA were identified. Then 30 men and 30 women were randomly selected from each quartile of BMD, resulting in a sample of 300 subjects. A few additional subjects with DNA were added. The association between the BsmI polymorphism in the *VDR* gene and various OA phenotypes in knees and hands was examined using analysis of covariance models in which the normalized residuals were used as the dependent variables. For description purposes, we also compute the least-squares means for the 4 hand OA (sums and proportion) and the 3 knee OA (sums) measurements in their original scale. For the hand OA measurements, the least-squares means were computed using analysis of covariance models in which we adjusted for sex and age. For the knee OA measurements, in addition to age and sex, we adjusted for body mass index and knee injury history. In these latter analyses, we had an observation for each knee and hence employed models that accounted for the potential correlation between the 2 observations for each subject (using Proc Mixed in SAS).

Heritability estimates for hand OA range from 28% to 34%, and power calculations indicate that we have 80% power to detect a quartile that accounts for 25% of the total variation of the trait. Since all heritability estimates for knee OA were less than this, we report no linkage results for this trait. We focus instead on association results for knee OA, where there is greater power to detect a relation between a particular allele and the trait.

RESULTS

The characteristics of the populations used in the linkage and association study are shown in Table 1. The average age for individuals in this study was about 60 years, with both sexes equally represented.

For linkage, we evaluated both cohort subjects and their children (subjects in the Offspring group). The results of linkage studies are shown in Figure 1. For each of the traits examined [including the proportion of hand joints affected with OA (K&L grade > 1), the sum of K&L grades for hands, sum of osteophyte scores in hands, and joint space narrowing in hand joints], there was no evidence of linkage with the VDR/COL2A1 locus (LOD score = 0). Similarly, knee OA showed no evidence of linkage to this location.

For association studies, we focused on the cohort subjects in whom we had evaluated polymorphisms at the BsmI site[30]. Table 2 shows the results of the association study between the *VDR BsmI* polymorphism and OA. For all of the phenotype definitions including sum of hand K&L scores, proportion of hand joints with OA, sum of hand osteophyte scores, sum of joint space narrowing scores, sum of K&L scores for knees, sum of knee osteophyte scores, and sum of joint space narrowing scores, there was no evidence of association.

Table 1. Description of subjects.

Measure	Linkage Study		Association Study	
	Number	Mean ± SD	Number	Mean ± SD
Age at hand measurement, yrs	1477	57.6 ± 10.8	283	59.7 ± 10.2
Age at knee measurement, yrs	1228	60.9 ± 12.1	312	72.6 ± 5.3
Sex, % male	1477	47	319	47
Body mass index, kg/m^2	1228	27.2 ± 4.9	294	25.5 ± 3.7
Subjects with previous knee injury, %	1228	10	278	9
Hand				
Sum of Kellgren-Lawrence grades	14723	7.9 ± 13.2	283	10.6 ± 15.8
Joints with OA, %	1472	9 ± 16	283	11 ± 17
Sum of osteophyte scores	1473	5.8 ± 9.6	283	7.0 ± 10.1
Sum of joint space narrowing scores	1472	2.7 ± 6.5	283	3.7 ± 8.4
Knee*				
Sum of Kellgren-Lawrence grades	1227	1.2 ± 1.7	310	1.9 ± 2.0
Sum of osteophyte scores	1112	1.5 ± 2.5	200	3.2 ± 3.5
Sum of joint space narrowing scores	1112	0.4 ± 1.0	200	1.0 ± 1.4

* Early cohort knee films were not read according to current scoring scheme for individual radiographic features and therefore, for some of the knee radiographs, scores for these features are not available.

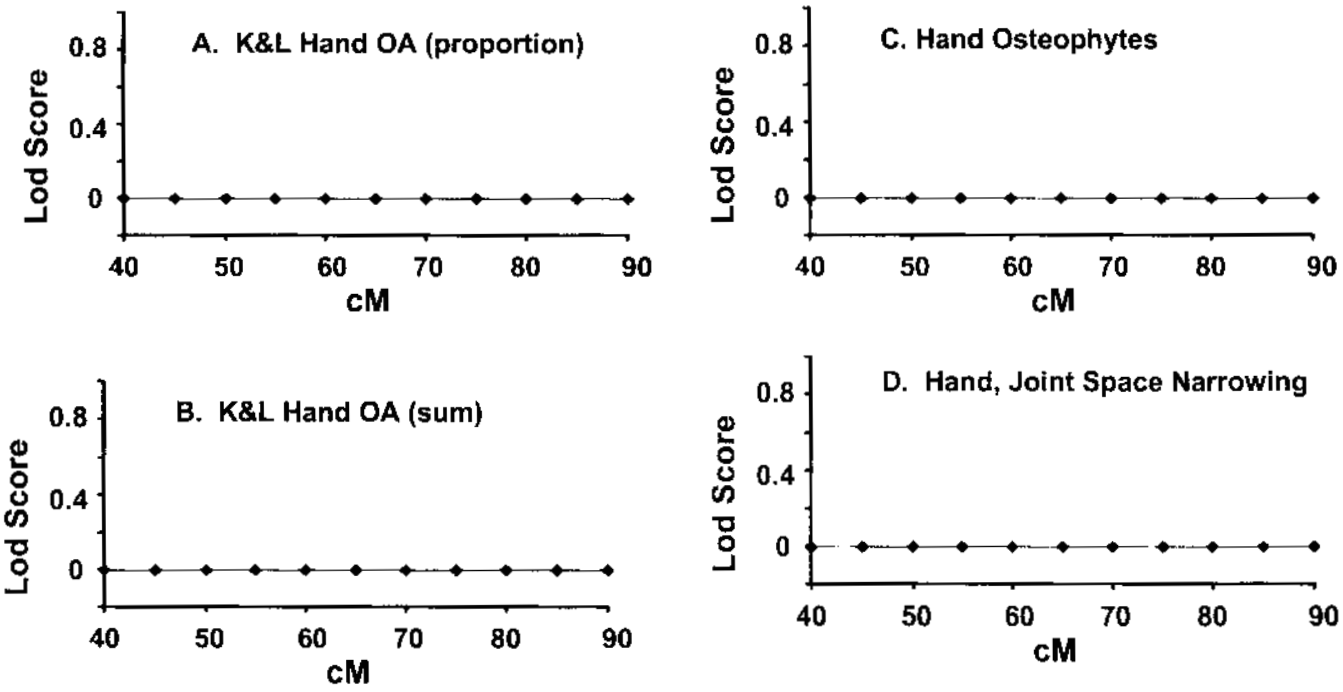

Figure 1. Results of multipoint LOD score analysis from the region flanking the VDR/COL2A1 for hand OA traits. Shown are multipoint analysis for proportion of hand joints affected with OA (A), sum of K&L scores for hand joints (B), sum of hand osteophyte scores (C), and sum of hand joint space narrowing scores (D). The VDR/COL2A1 locus is located about 65 cM from the p-ter of chromosome 12.

DISCUSSION

We found no evidence of association or linkage between the COL2A1/VDR locus on human chromosome 12q and OA in a series of 296 well studied extended families in the Framingham Heart Study. Our results, which are the first to use powerful linkage approaches in multiple families from the community, strongly suggest that the mutations or polymorphisms in *VDR* and *COL2A1* genes are not a major cause of common types of OA. We also tested allelic association of a *VDR* polymorphism with the occurrence of OA in a subsample of participants in the original cohort of the Framingham Study.

The VDR association with OA has been among the most intriguing recently identified, especially given our findings[33]

Table 2. Association between the VDR BsmI polymorphism and OA according to site and feature. Mean ± standard error of grades/scores adjusted for sex and age of hand OA measures. For knee OA, adjusted for age at knee radiograph, body mass index, and knee injury history.

Measure of Radiographic Disease	BB	Bb	bb	p
Hand				
Sum of Kellgren-Lawrence grades	10.27 ± 2.03	9.57 ± 1.07	11.82 ± 1.25	0.59
Proportion of joints with OA	0.10 ± 0.02	0.10 ± 0.01	0.12 ± 0.01	0.84
Sum of osteophyte scores	7.04 ± 1.29	6.12 ± 0.68	8.18 ± 0.80	0.26
Sum of joint space narrowing scores	2.72 ± 1.19	3.30 ± 0.63	4.39 ± 0.73	0.77
Knee				
Sum of Kellgren-Lawrence grades	1.06 ± 0.15	0.86 ± 0.08	1.04 ± 0.09	0.55
Sum of osteophyte scores	1.91 ± 0.35	1.42 ± 0.19	1.85 ± 0.21	0.27
Sum of joint space narrowing scores	0.49 ± 0.14	0.49 ± 0.08	0.56 ± 0.09	0.79

that vitamin D status may affect OA progression. Nevertheless, the association of VDR alleles with OA appears inconsistent[18-25]. This may be due to differences in the relative importance of this gene in different populations, differences in environmental factors related to vitamin D metabolism, or the presence of other genetic factors that influence VDR function. A Dutch study[18] suggested that *VDR* polymorphisms may be specifically associated with osteophyte development, and a study of spinal disease[21] also reported an association of osteophytes with allelic variation in the VDR. We found no evidence for an association of the *VDR* gene with osteophytes.

The Framingham Heart Study Cohort represents a unique opportunity to study OA. The original cohort, composed of 5209 subjects, was recruited in 1948 as a representative sample of Framingham, Massachusetts, USA, and participants have been examined every 2 years since. Their children joined the offspring study in 1971 and have been followed every 4 years. Thus, a considerable amount of information about disease risk factors is available. Further, at the time the study began, individuals were healthy and development and progression of disease has been followed. This significantly reduces a selection bias in recruitment of subjects and supports the view that the majority of FHS subjects exhibit common forms of disease.

We recognize that our study may have insufficient power to detect a QTL through linkage analyses or association studies. However, we found that all families have LOD scores of 0 and that the differences in OA phenotypes between the BsmI genotype groups are extremely small. Hence, there is no evidence of a QTL in this region in our data. Our study of the BsmI polymorphism and OA was as large as other studies[19,21] that have shown associations of OA with *VDR*. Further, our results do not likely reflect insufficient power, as we found no suggestive relationship with *VDR*.

The heritability of hand OA in the FOS is high in this cohort, and results of a segregation analysis[27] for generalized OA (also highly heritable) suggest the presence of a Mendelian recessive gene with a multifactorial component, representing either polygenic or environmental factors. Therefore, mapping and identification of OA associated genes is feasible. One strength of our study is that we examined multiple phenotypes and have phenotypic information for large, extended families taken when parents and offspring are at a similar age. While OA associated genes are likely to exist, the results of the analyses reported here suggest that the *COL2A1*/*VDR* genes are not major factors contributing to common OA.

REFERENCES

1. Altman RD, Bloch DA, Bole GG Jr, et al. Development of clinical criteria for osteoarthritis. J Rheumatol 1987;14:3-6.
2. Adams P, Benson V. Current estimates from the National Health Interview Survey, 1991. Vital and health statistics series 10: Data from the National Health Survey 1992;184:1-232.
3. Badley E, Rasooly I, Webster G. Relative importance of musculoskeletal disorders as a cause of chronic health problems, disability, and health care utilization: findings from the 1990 Ontario Health Survey. J Rheumatol 1994;21:505-14.
4. Kellgren JH, Lawrence JS, Bier F. Genetic factors in generalized osteoarthritis. Ann Rheum Dis 1963;22:237-55.
5. Felson DT, Hannan MT, Naimark A, et al. Occupational physical demands, knee bending, and knee osteoarthritis: results from the Framingham Study. J Rheumatol 1991;18:1587-92.
6. Chaisson C, Zhang Y, McAlindon T, Hannan M, Felson D. Risk factors for radiographic hand osteoarthritis: The Framingham Study [abstract]. Arthritis Rheum 1996;39 Suppl:S300.
7. Felson D, Zhang Y, Hannan M, et al. Risk factors for incident radiographic knee osteoarthritis in the elderly: the Framingham Study. Arthritis Rheum 1997;40:728-33.
8. Leppavuori J, Kujala U, Kinnunen J, et al. Genome scan for predisposing loci for distal interphalangeal joint osteoarthritis: evidence for a locus on 2q. Am J Hum Genet 1999;64:1060-7.
9. Loughlin J, Mustafa Z, Irven C, et al. Stratification analysis of an osteoarthritis genome screen — suggestive linkage to chromosomes 4, 6 and 16. Am J Hum Genet 1999;65:1795-8.
10. Chapman K, Mustafa Z, Irven C, et al. Osteoarthritis-susceptibility locus on chromosome 11q. Am J Hum Genet 1999;65:167-74.
11. Williams C, Jimenez S. Heredity genes and OA. Rheum Dis Clin N Am 1993;19:523-43.

12. Byers PH. Molecular genetics of chondrodysplasias, including clues to development, structure, and function. Curr Opin Rheumatol 1994;6:345-50.
13. Baldwin C, Farrer L, Adair R, Dharmavaram R, Jimenez S, Anderson L. Linkage of early-onset osteoarthritis and chondrocalcinosis to human chromosome 8q. Am J Hum Genet 1995;56:692-7.
14. Vikkula M, Olsen BR. Unravelling the molecular genetics of osteoarthrosis. Ann Medicine 1996;28:301-4.
15. Rimoin DL. Molecular defects in the chondrodysplasias. Am J Med Genet 1996;63:106-10.
16. Cicuttini F, Spector T. Genetics of osteoarthritis. Ann Rheum Dis 1996;55:665-7.
17. Ritvaniemi P, Korkko J, Bonaventure J, et al. Identification of COL2A1 gene mutations in patients with chondrodysplasias and familial osteoarthritis. Arthritis Rheum 1995;38:999-1004.
18. Uitterlinden AG, Burger H, Huang Q, et al. Vitamin D receptor genotype is associated with radiographic osteoarthritis at the knee. J Clin Invest 1997;100:259-63.
19. Keen RW, Hart DJ, Lanchbury JS, Spector TD. Association of early osteoarthritis of the knee with a Taq I polymorphism of the vitamin D receptor gene. Arthritis Rheum 1997;40:1444-9.
20. Meulenbelt I, Bijkert C, DeWildt SC, et al. Haplotype analysis of three polymorphisms of the COL2A1 gene and associations with generalized radiological osteoarthritis. Ann Hum Genet 1999;63:393-400.
21. Jones G, White C, Sambrook P, Eisman J. Allelic variation in the vitamin D receptor, lifestyle factors and lumbar spinal degenerative disease. Ann Rheum Dis 1998;57:94-9.
22. Uitterlinden AG, Burger H, van Duijn CM, et al. Adjacent genes, for COL2A1 and the vitamin D receptor, are associated with separate features of radiographic osteoarthritis of the knee. Arthritis Rheum 2000;43:1456-64.
23. Aerssens J, Dequeker J, Peeters J, Breemans S, Boonen S. Lack of association between osteoarthritis of the hip and gene polymorphisms of VDR, COL1A1, and COL2A1 in post-menopausal women. Arthritis Rheum 1998;41:1946-50.
24. Loughlin J, Sinsheimer JS, Mustafa Z, et al. Association analysis of the vitamin D receptor gene, type I collagen gene COL1A1, and the estrogen receptor gene in idiopathic osteoarthritis. J Rheumatol 2000;27:779-84.
25. Huang J, Ushiyama T, Inoue K, Kawasaki T, Hukuda S. Vitamin D receptor gene polymorphisms and osteoarthritis of the hand, hip and knee: a case-control study in Japan. Rheumatology 2000; 9:79-84.
26. Dawber TR, Kannel WB, Lyell LP. An approach to longitudinal studies in a community: The Framingham Study. Ann NY Acad Sci 1963;107:539-56.
27. Felson D, Couropmitree N, Chaisson C, et al. Evidence for a mendelian gene in a segregation analysis of generalized radiographic osteoarthritis: The Framingham Study. Arthritis Rheum 1998;41:1064-71.
28. Kellgren J, Lawrence J. Radiological assessment of osteoarthritis. Ann Rheum Dis 1957;16:494-502.
29. Felson DT, McAlindon TE, Anderson JJ, et al. Defining radiographic osteoarthritis for the whole knee. Osteoarthritis Cart 1997;5:241-50.
30. Kiel DP, Myers RH, Cupples LA, et al. The BsmI vitamin D receptor restriction fragment polymorphism (bb) influences the effect of calcium uptake on bone mineral density. J Bone Miner Res 1997;12:1049-57.
31. Almasy L, Blangero J. Multipoint quantitative-trait linkage analysis in general pedigrees. Am J Hum Genet 1998;62:1198-211.
32. Fulker DW, Cherny SS, Sham PC, Hewitt JK. Combined linkage and association sib-pair analysis for quantitative traits. Am J Hum Genet 1999;64:259-67.
33. McAlindon TE, Felson DT, Zhang Y, et al. Relation of dietary intake and serum levels of vitamin D to progression of osteoarthritis of the knee among participants in the Framingham Study. Ann Intern Med 1996;125:353-9.