

ASSOCIATION OF EARLY OSTEOARTHRITIS OF THE KNEE WITH A *Taq I* POLYMORPHISM OF THE VITAMIN D RECEPTOR GENE

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Objective. To determine whether a polymorphism of the vitamin D receptor (VDR) gene, already associated with osteoporosis, might also relate to the risk of osteoarthritis (OA).

Methods. A population cohort of 351 postmenopausal women (ages 45–64 years) was studied using anteroposterior radiographs of the knee, which were graded for OA according to the Kellgren and Lawrence classification system. The VDR genotype was determined by using polymerase chain reaction and by digestion with the restriction enzyme *Taq I*.

Results. The VDR allele "T" was associated with an increased risk of knee OA compared with the "t" allele, with an odds ratio of 2.82 (95% confidence interval 1.16–6.85; $P = 0.02$). A dominant pattern of risk was suggested. The frequency of the VDR genotype differed significantly between OA cases and controls ($P = 0.03$ by Fisher's exact test).

Conclusion. A *Taq I* polymorphism of the VDR gene appears to be associated with an increased risk of knee OA. This is the first genetic locus that has been shown to influence the risk of early knee OA within the general population.

Osteoarthritis (OA) is a common age-related and chronic skeletal condition that is associated with considerable morbidity and mortality, although at present the pathogenesis of this condition remains largely unknown. Many environmental factors and other independent conditions have been associated with OA, including

obesity (1,2), previous injury and/or meniscectomy (3), knee-bending occupations (4), smoking (5,6), sex hormones and gynecologic disorders (6,7), and other metabolic factors (8,9). Family studies have suggested a strong genetic component to OA, with higher prevalence rates in first-degree relatives of index cases (10). A recent population-based UK study of twins has also demonstrated a clear genetic effect for radiologic OA of the knee and hand in women, with up to 65% of the variance being explained by genetic factors (11).

OA of the knee and hip appears to be protective for osteoporotic fractures, with OA cases having a 5–10% greater bone mass compared with controls (12). There is also evidence that OA is associated with underlying abnormalities in bone structure and mineralization, findings that are independent of body weight and skeletal loading (13). Since family and twin studies have demonstrated a strong genetic component to both OA (9,10) and osteoporosis (14,15), we have hypothesized that common genetic factors may influence the development and/or protection against both conditions. We therefore have examined whether polymorphisms of the vitamin D receptor (VDR) gene, which were recently associated with low bone mineral density (BMD) in twin and population studies (15,16), were also associated with an altered risk of OA in a large population of normal, white women.

PATIENTS AND METHODS

The study design was a nested case-control study involving a population cohort of 1,003 women, who had a mean \pm SD age of 54.2 ± 6.0 years. Women in the age range 45–64 years had been selected from a large (total of 11,000 registered patients) single general practice in Chingford, a district of northeast London, to participate in a longitudinal epidemiologic study of rheumatic diseases. We identified 1,353 women within the age range specified, and of these, 78% (1,003) agreed to participate in our study. The area is predominantly middle class, 98% of residents are white, and the characteristics of the population are similar to those of the general UK population in terms of height, weight, smoking

Supported in part by the Arthritis and Rheumatism Council (ARC) and the St. Thomas' Hospital Special Trustees. Dr. Keen is the recipient of an ARC Clinical Fellowship (K0504).

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Submitted for publication September 11, 1996; accepted in revised form March 17, 1997.

Table 1. Demographic variables for the cohort of 351 women, by their knee osteoarthritis (OA) status*

Variable	Controls			OA cases			
	Total (n = 269)	Grade 0 (n = 201)	Grade 1 (n = 68)	Total (n = 82)	Grade 2 (n = 63)	Grade 3 (n = 15)	Grade 4 (n = 4)
Age, years	54.4 ± 4.8	54.1 ± 4.8	55.4 ± 4.6	58.3 ± 4.7†	58.1 ± 4.6	58.1 ± 5.2	62.5 ± 1.9
BMI, kg/m ²	25.0 ± 3.6	24.5 ± 3.5	26.2 ± 3.8	28.0 ± 4.0†	27.7 ± 3.7	28.4 ± 4.5	30.8 ± 4.9
Age at menopause, years	47.8 ± 5.0	47.9 ± 4.9	47.1 ± 5.3	47.7 ± 4.7	47.7 ± 4.8	48.1 ± 4.8	46.5 ± 4.1
Ever use of HRT, no. (%)	78 (29)	57 (28)	21 (31)	15 (18)‡	10 (16)	5 (33)	0 (0)
Ever smokers, no. (%)	112 (42)	83 (41)	29 (43)	37 (45)	29 (46)	6 (40)	2 (50)
BMD femoral neck, gm/cm ²	0.73 ± 0.12	0.73 ± 0.11	0.75 ± 0.12	0.77 ± 0.13§	0.77 ± 0.14	0.78 ± 0.10	0.72 ± 0.08

* Unless otherwise indicated, values are the mean ± SD. BMI = body mass index; HRT = hormone replacement therapy; BMD = bone mineral density.

† $P < 0.001$ versus controls.

‡ $P = 0.05$ versus controls.

§ $P = 0.008$ versus controls.

status, hysterectomy rates, and use of hormone replacement therapy (HRT) (2). Women were selected for this study on the basis of postmenopausal status, which was defined by the absence of menstruation for 12 months and confirmed by measurement of sex hormone levels (serum estradiol, luteinizing hormone, and follicular-stimulating hormone). All women completed an extensive questionnaire that detailed risk factors for both osteoporosis and OA. The study was approved by the local ethics committee, and all women gave their informed consent to participate.

Anteroposterior weight-bearing knee radiographs were obtained using standard procedures. The radiographs were subsequently graded by a single blinded observer (DJH) according to the methods of Kellgren and Lawrence, in which a scale of 0–4 is used (0 representing no disease and 4 representing severe disease) (17). We used a radiologic definition of OA that is the currently accepted standard for epidemiologic studies of OA in populations (18). Knee OA was defined as present if a grade of ≥ 2 was given, while controls were classified as having no OA if they were given a grade of ≤ 1 . The intra-observer reproducibility for this technique was good, with a kappa score of >0.8 . Presence of Heberden's nodes was determined by clinical hand examination (19). BMD was measured at the femoral neck using dual x-ray absorptiometry with a Hologic QDR-1000 (Hologic, Waltham, MA). Reproducibility (% coefficient of variation), assessed by duplicate measures in healthy volunteers, was 1.4% at the femoral neck.

DNA was extracted from peripheral blood leukocytes using standard techniques. Polymerase chain reaction (PCR) was used to amplify a 740-basepairs fragment of the VDR gene using published primers and reaction conditions (15,16). VDR genotypes were obtained by digestion of the PCR product with the restriction enzyme *Taq I* (Promega, Madison, WI), and alleles were coded as "T" (absence of *Taq I* restriction site) and "t" (presence of restriction site).

Differences in demographic variables between OA cases and controls, and between VDR genotypes, were initially compared using analysis of variance and chi-square test. VDR genotype frequencies were compared between OA and control groups using Fisher's exact test. Conditional logistic regression analysis was used to estimate the odds ratio (OR) and 95%

test-based confidence intervals (95% CI) for developing a radiologic feature of OA with the individual VDR genotypes. The homozygous VDR genotype "tt" was set as baseline. Adjustment for other potential confounding variables was performed using conditional logistic regression with the statistical software program STATA (Stata, College Station, TX).

RESULTS

Among the total study cohort, 501 women were identified as postmenopausal and received graded knee radiographs. Full genotype results were available for a group of 351 women (70%), comprising 82 subjects with knee OA and 269 controls with normal-appearing radiographs (mean ± SD age 55.3 ± 5.0 years). Subdivision of this group of women according to radiologic grading for OA showed that, among the OA cases, 63 (76.8%) were grade 2, 15 (18.3%) were grade 3, and 4 (4.9%) were grade 4. Among the controls, 201 (74.7%) were grade 0 and 68 (25.3%) were grade 1. The characteristics of the women according to the absence or presence of knee OA are shown in Table 1, and are similar to those of the whole cohort except for age, menopause status, and duration of menopause. No significant differences were observed between the women for whom we had genotype results and those for whom the DNA was unavailable. Within the group of 351 women for whom we had full results, there were significant differences between the OA cases and controls in terms of potential confounders such as age, body mass index (BMI), use of HRT, and hip BMD.

VDR genotype frequencies in the group of 351 women were similar to those reported in other white populations, and were in Hardy-Weinberg equilibrium (15,16). There were no significant differences in the baseline characteristics (Table 1) among the different

Table 2. Vitamin D receptor (VDR) genotype frequencies according to the Kellgren and Lawrence grading system for osteoarthritis*

VDR genotype	Grade 0 (n = 201)	Grade 1 (n = 68)	Grade 2 (n = 63)	Grade 3 (n = 15)	Grade 4 (n = 4)
TT	74 (36.8)	24 (35.3)	19 (30.2)	10 (66.7)	0 (0)
Tt	87 (43.3)	36 (52.9)	38 (60.3)	4 (26.7)	4 (100)
tt	40 (19.9)	8 (11.8)	6 (9.5)	1 (6.6)	0 (0)

* Values are the no. (%) of subjects with each genotype. On the Kellgren and Lawrence scale, grade 0 represents no disease and grade 4 represents severe disease.

VDR genotype groups within this total group or after stratification by Kellgren and Lawrence OA grade. The frequencies of the *Taq* I genotypes were found to differ significantly between the OA cases and controls using Fisher's exact test ($P = 0.03$). The "tt" genotype frequency was reduced with worsening OA status, although the number of cases of severe OA (grade ≥ 3) was too small for formal analysis (Table 2). The frequency of the "T" allele was increased by 5% in the OA group compared with the controls, although this difference was not significant.

The OR for knee OA in the "TT" VDR genotype group was 2.42 (95% CI 0.94–6.21; $P = 0.07$) and, in the group with the heterozygous genotype "Tt," the OR was 3.15 (95% CI 1.26–7.83; $P = 0.01$), both compared with the alternate homozygous genotype "tt" group (Table 3). After adjustment for variables judged to be potential confounders (age, BMI, HRT use, and hip BMD), the OR for knee OA in those with the "TT" genotype (versus the "tt" group) remained marginally increased at 2.82 (95% CI 0.98–8.10; $P < 0.05$). After similar adjustment, the OR for those with the "Tt" genotype (versus the "tt" group) was marginally reduced at 2.98 (95% CI 1.09–8.12; $P = 0.03$). Because the risk of knee OA appeared to be associated with the presence of the "T" allele with a dominant inheritance pattern, analysis was subsequently performed on the genotype groups "TT" and "Tt" combined against the "tt" homozygous group set at baseline. Crude analysis showed an OR of 2.82 (95% CI 1.16–6.85; $P = 0.02$) for the risk of knee OA in association with the "T" allele. This increase in risk

remained after adjustment for variables as previously detailed, with an OR of 2.60 (95% CI 1.01–6.71; $P < 0.05$).

No significant relationship was observed between VDR genotype and nodal arthritis, as determined by the presence of Heberden's nodes. In addition, there was no difference in genotype frequencies in the subset of subjects with knee OA who also had clinical nodes present compared with those with knee OA alone (data not shown). Within the total group of 351 women, we were also not able to demonstrate any overall significant relationship between BMD and VDR genotype, although there was a 4.6% difference in BMD at the hip between the homozygous genotypes "TT" and "tt." Adjustment for potential confounders such as age, BMI, HRT use, and OA grade did not appreciably alter these findings.

DISCUSSION

Our data demonstrate an association between a *Taq* I polymorphism of the VDR locus and early knee OA in women from the general population. The "T" allele, which has previously been associated with high bone mass, was associated with a nearly 3-fold increased risk for development of knee OA when compared with the alternate allele. The pattern of risk was observed to be co-dominant, with the homozygous genotype "TT" and the heterozygous genotype "Tt" groups both having an equivalent increased risk of knee OA. This relationship cannot be explained on the basis of age, since we observed no significant deviation from expected genotype frequencies in the total group after stratification by age. This relationship was also independent of other factors such as BMI, HRT use, and BMD, which have been shown to influence OA risk and were found to differ between the OA cases and controls. Our finding that there was no relationship between the VDR locus and nodal arthritis suggests that differing genetic mechanisms may underlie the development of Heberden's nodes and associated generalized OA. We had insufficient numbers of cases with severe knee OA and marked

Table 3. Effect of vitamin D receptor (VDR) genotype on risk of knee osteoarthritis (OA)

VDR genotype	Frequency in OA cases, no. (%)	Frequency in controls, no. (%)	Odds ratio, crude (95% confidence interval)	Odds ratio, adjusted (95% confidence interval)*
TT	29 (35.4)	98 (36.4)	2.42 (0.94–6.21)	2.82 (0.98–8.10)
Tt	46 (56.1)	123 (45.7)	3.15 (1.26–7.83)	2.98 (1.09–8.12)
tt	7 (8.5)	48 (17.9)	1.0	1.0

* Adjusted for age, body mass index, femoral neck bone mineral density, and use of hormone replacement therapy.

joint space narrowing to accurately compare the genetic findings between those having either osteophytic or cartilage loss phenotypes, although the "T" allele frequency was increased in the 19 subjects with severe OA.

We were also not able to demonstrate any significant overall effect of VDR genotype on BMD, although previous work has indicated that sample sizes would need to be larger than 300 for significant relationships to be demonstrated, assuming an increased locus-specific disease risk of 40% (20). Our data can therefore exclude an effect for the VDR locus on osteoporosis risk of this magnitude, although weaker effects would obviously require larger studies. Other studies have shown that dietary intake of calcium may modify the effect of VDR genotype on BMD, and this may also have confounded our results (21,22).

It has been proposed that the increase in bone density observed in OA indicates that this disease might initially be a subchondral bone disorder rather than a defect in cartilage, with an increase in subchondral stiffness (23). This would make the subchondral bone less deformable to impact loading and would result in more force being transmitted to overlying tissue, thereby predisposing to articular cartilage loss. Our finding that the increase in OA risk associated with VDR genotype was independent of BMD suggests, however, that the molecular mechanisms are likely to be more complex than a simple direct action on bone strength with consequent cartilage damage. Serum levels and dietary intake of vitamin D have been shown to correlate with the progression of knee OA, with this relationship being independent of any effect of vitamin D on BMD (24). Vitamin D has been shown to stimulate synthesis of proteoglycan by mature articular cartilage *in vitro* (25), and this suggests that vitamin D, through its receptor, may directly affect articular cartilage metabolism. Vitamin D also has effects on the immune system, and altered function of the VDR may result in immunomodulation (26). This may be relevant, since low levels of inflammation have been associated with knee OA (27,28). The *Taq I* polymorphism represents a synonymous codon change (16), and this suggests that for the receptor's function or structure to be affected, there must be linkage between the *Taq I* site and other sequence changes within the VDR gene. Although an early study provided some *in vitro* evidence for either altered transcription or messenger RNA stability (16), this has yet to be confirmed.

Alternatively, the VDR gene may not be the "disease locus" itself, but may be in linkage disequilibrium with a nearby novel susceptibility locus. This has

been proposed as one explanation for the differing results that have been observed between VDR genotype and BMD, since the degree of linkage disequilibrium may differ between population groups (29). Families of genes with related function often map to the same chromosomal location, and our finding that the *Taq I* polymorphism is a marker for knee OA suggests that genes with an effect on both bone and cartilage metabolism may also map to this region. The VDR locus maps to the chromosome 12q12-14 region by somatic cell hybridization, and potential candidate genes mapping to chromosome 12q include the type II procollagen (COL2A1) gene and insulin-like growth factor type I gene, both of which have been implicated in OA and osteoporosis (30,31). The COL2A1 gene maps within 920 kilobases from the VDR locus (32), and mutations of this gene have been demonstrated in familial forms of OA and chondrodysplasias (31). These mutations are, however, rare and unlikely to account for the majority of clinical cases observed in the general population.

To date, several studies of association have examined the relationship between the COL2A1 locus and OA in the general population. A small study of 86 women (all <60 years of age) with symptomatic and radiologic OA in more than 1 joint showed an association with a *Bam HI* restriction site in COL2A1, with the heterozygote frequency being increased in cases compared with controls (22% versus 11%) (33). A further case-control study failed, however, to demonstrate any significant association between either a biallelic *Pvu II* polymorphism or a multiallelic variable number tandem repeat (VNTR) of the COL2A1 gene and generalized OA in 41 patients (34). In that study, there was also no relationship between these 2 markers and OA of the finger in an additional group of 49 subjects. Affected sibling-pair analysis has failed to show any significant linkage between the COL2A1 locus and nodal generalized OA, with no evidence of excess allele sharing of either 5 COL2A1 biallelic polymorphisms in 21 sibling pairs (*Hind III*, *Bam HI*, and 3 *Pvu II* sites) or the COL2A1 VNTR in 38 sibling pairs (35,36). However, affected sibling-pair analysis in a small sample of 99 subjects has suggested a possible linkage to a region of chromosome 2q with nodal arthritis (37). Candidate genes mapping to this region include those for the $\alpha 2$ chain of type V collagen, interleukin-8 receptor, and the $\alpha 1$ chain of fibronectin, although, to date, none of these loci has been formally tested.

At present, all these studies have had inadequate power to detect linkage for loci having modest effects on susceptibility to, and/or severity of, OA. In these circum-

stances, tests of association in large populations may be more appropriate (38). Studies of association may, however, be confounded by population admixture, heterogeneity, or stratification, and it is important that preliminary findings are replicated in a separate cohort. Preliminary results from a large population cohort in the Netherlands appear to confirm our findings in that they have indicated an association between the VDR locus and osteophytes in OA at the knee (39), and would support our hypothesis that a novel, linked gene maps to this region of chromosome 12. Additional and larger studies will be required, however, to confirm whether this observed relationship between VDR genotype and early knee OA is seen in more severe cases, and also whether this effect is manifest at other skeletal sites. Longitudinal studies may also determine whether this locus has any influence on the progression of joint damage at either the knee or other sites.

In unrelated subjects, linkage disequilibrium extends over short genetic distances (1–2 mega-bases), and our data suggest that future work should be targeted to characterize novel genes flanking the VDR locus. These loci can then be tested for an association with the risk of OA, and mutation screening and sequence analysis will subsequently allow the candidacy of these loci to be fully tested and the physiologic function to be determined. This work also suggests that given the relationship between OA and osteoporosis, future studies that test candidate genes for one condition should also investigate their association against the other.

In summary, this study has shown a relationship between early knee OA and a *Taq I* polymorphism of the VDR gene on chromosome 12. The risk of knee OA is increased nearly 3-fold in those with the VDR genotypes "TT" and "Tt" when compared with those with the "tt" genotype. Further work is required to characterize whether this relationship is due specifically to alterations in the VDR protein and its function, or whether it is because of linkage disequilibrium between the *Taq I* polymorphism and a novel disease gene. Identification of the precise molecular mechanisms associated with these findings will provide valuable insight into the etiology underlying both OA and osteoporosis, and may hopefully improve our treatment of these common and disabling conditions.

ACKNOWLEDGMENTS

We acknowledge and thank Dr. David Doyle and all the staff and women of the Chingford study, Highams Park Medical Practice, Gabriela Surdulescu for laboratory assis-

tance, and Dr. Paul Kelly and Dr. Nigel Morrison for their comments on the manuscript.

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