Vitamin D Receptor Genotype Is Associated with Radiographic Osteoarthritis at the Knee

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Abstract

Osteoporosis and osteoarthritis are age-related disorders of the skeleton with genetic components. Low bone density is a risk factor for osteoporotic fracture while osteoarthritis is associated with increased bone density. The 1,25-dihydroxyvitamin D_3 receptor (VDR) gene locus was previously found to be associated with bone density. We therefore studied the relationship between radiographic osteoarthritis at the knee and VDR genotype in a population-based sample ($n = 846$), using molecular haplotyping of anonymous intragenic DNA polymorphisms. Radiographic osteoarthritis was defined using the Kellgren score, which is based on the assessment of osteophytes and joint space narrowing (JSN). We show that one VDR haplotype allele is significantly overrepresented in individuals with knee osteoarthritis and associated with a 2.27-fold increased relative risk (95% confidence interval 1.46, 3.52). Adjustment for bone density at the femoral neck did not change these results, indicating that the association is not mediated by bone density. The association appeared to be largely explained by the presence of osteophytes rather than JSN. Our results indicate a role of the VDR gene in the pathogenesis of osteophytes while linkage disequilibrium with another nearby gene, i.e., the collagen type IIa1 gene encoding the most abundant protein in cartilage, might contribute to the association. (*J. Clin. Invest.* 1997. 100:259–263.) Key words: genetic • osteophytes • Kellgren score • epidemiology • haplotype

Introduction

Osteoporosis and osteoarthritis are common age-related chronic disorders of the skeleton, involving deterioration of bone and cartilage tissue, respectively. Apart from being influenced by environmental factors, both osteoporosis (1, 2) and osteoarthritis (3, 4) have a strong genetic component as demonstrated by family and twin studies. Remarkably, severe forms of these frequent disorders rarely coexist in one patient (5–7).

Low bone density is an essential feature of osteoporosis (8), while it has been shown that bone density is increased in subjects with osteoarthritis (9, 10). Several studies have demonstrated that genetic variants of the gene locus encoding the receptor for the hormonally active form of vitamin D (1,25-dihydroxyvitamin D_3), i.e., the vitamin D receptor (VDR)[1] gene locus on chromosome 12, is associated with bone density (11, 12). In light of the above we have analyzed the association between VDR genotype and radiographic osteoarthritis at the knee in a large population-based study of 846 men and women. To determine genetic variants of the VDR gene locus we have developed a direct molecular haplotyping PCR procedure (13) to simultaneously monitor three adjacent RFLPs (BsmI, ApaI, TaqI) in relation to each other. By discriminating three common haplotype alleles (1 = baT, 2 = BAt, 3 = bAT) this genotyping procedure results in higher genetic resolution. Radiographic osteoarthritis (ROA) is commonly defined using the Kellgren score (3). This score is a composite score largely based on the assessment of two different characteristics, i.e., osteophytes and narrowing of the joint space. Osteophytes are osseous and cartilaginous neoplastic protrusions forming mostly at the margin of osteoarthritic joints while joint space narrowing (JSN) is thought to be due to degeneration of cartilage. Presence of osteophytes or JSN in a given individual does not always imply presence of ROA as scored using the Kellgren score. We therefore analyzed these composite features of ROA separately in relation to VDR genotype, and independently of the presence of ROA.

Methods

Study population. This study was conducted as part of the Rotterdam Study, a prospective population-based cohort study of determinants and prognosis of chronic diseases in the elderly (14). Eligible for the study were all inhabitants aged 55 yr or over of the district of Ommoord in Rotterdam, The Netherlands. A total of 10,275 persons, of whom 9,161 (89%) were living independently, were invited for the study. In the independently living population, the overall response rate was 77% for the home interview and 71% for the examination in

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1. *Abbreviations used in this paper:* bmi, body mass index; JSN, joint space narrowing; ROA, radiographic osteoarthritis; VDR, 1,25 dihydroxyvitamin D_3 receptor.

a research center. Written informed consent was obtained from each participant. The Rotterdam Study has been approved by the Medical Ethics Committee of Erasmus University Medical School. The analysis of the association between VDR genotype and ROA was performed in a sample from the Rotterdam Study. From all 5,931 independently living participants with bone density measurements, 1,453 subjects were excluded according to the following criteria: older than 80 yr; use of a walking aid; known diabetes; use of cytostatics, thyroid hormone, or diuretics. From the 4,478 remaining subjects, an age-stratified random sample of 1,000 men and 1,000 women was drawn with balanced numbers (200) in five-yr age-categories. By the time of this analysis, VDR genotyping and scoring of x-rays of knees of 405 men and 441 women had been completed.

Osteoarthritis and bone mineral density measurement. Osteoarthritis of the knee was assessed as described previously (10, 15). Briefly, anteroposterior weight-bearing x-rays of the knees were graded for osteoarthritis on a five point scale (0–4) according to Kellgren (16). Definite ROA was defined as Kellgren score ≥ 2 at one or both sides of the knee. We scored separate features of ROA (osteophytes and JSN) independently of the presence of ROA as scored using the Kellgren score. Osteophytes were scored separately at both knees at the lateral, medial, and central femur and tibia on a four point scale (0–3) and added up to a total score. Definite presence of osteophytes was defined as having a total score of ≥ 6, which corresponded roughly with the upper quartile of the population. The knee joint space was measured in millimeters at the medial and lateral sides of both knees. Definite JSN was defined as having a total sum of 20 mm or less at the four locations combined, which corresponded roughly with the lowest quartile of the population. For 544 of the 846 subjects (64%) measurements of the joint space were available. Bone density (grams/square centimeter) at the femoral neck was measured by dual energy x-ray absorptiometry as described previously (17).

Genotyping procedures. Three anonymous polymorphic restriction enzyme recognition sites at the 3′ end of the VDR gene, BsmI, ApaI, and TaqI (10, 11) were assessed in relation to each other by a direct molecular haplotyping PCR procedure which we developed (13). The alleles were named similarly as previously described for alleles defined by individual RFLPs (18, 19): in genotypes such as "BAt-baT" capitals denote absence and lower case letters denote presence of the site for the restriction enzymes BsmI (B/b), ApaI (A/a), and TaqI (T/t) on each of the alleles. The haplotype alleles were coded 1–5 in order of decreasing frequency in the population; genotypes are presented as combinations of two alleles in square brackets (1 = baT, frequency = 48%; 2 = BAt, frequency = 40%; 3 = bAT, frequency = 11%). Detailed information on haplotype alleles and genotype frequencies in a larger sample from the Rotterdam Study, including this population, can be found elsewhere (13).

Analysis. Individuals were grouped according to VDR genotype. Alleles 4 and 5 have a population frequency of $< 1\%$ and were excluded from analysis. Rare VDR genotypes, i.e., $n < 5$ in men and women combined, were also excluded from the analyses. Frequencies of VDR alleles among different subgroups were compared by the χ^2 test. Differences in the prevalence of Kellgren 2+, definite osteophytes, and definite JSN were tested for statistical significance using the χ^2 test. The strength of the association between VDR genotype and ROA was estimated by multiple logistic regression and expressed as odds ratios, presented with 95% confidence intervals. To assess the contribution of particular alleles in the association, individuals carrying at least one copy of that allele were combined and compared with the reference group consisting of individuals carrying no copy of the allele. All analyses were carried out while adjusting for age and body mass index (bmi). The analyses of the association between VDR genotype and ROA were additionally adjusted for bone mineral density at the femoral neck to examine whether the relationship is independent of bone density.

Results

Baseline characteristics of the population are presented in Table I. VDR genotype frequencies, and prevalence according to VDR genotype of ROA according to the Kellgren score, osteophytes and JSN are presented in Table II. Substantial differences were observed among the VDR genotypes in the prevalence of ROA as defined by having a Kellgren score of ≥ 2 which was significant when men and women were combined. Similar differences were observed when the presence of osteophytes was assessed independently of the presence of ROA. No allele dose effect could be observed for allele 1 while the absence of cases in the rare homozygote for allele 3 prevented a conclusion about an allele dose effect. Although significant differences among VDR genotypes in JSN were observed in men, this could not be seen for women. When men and women were combined, no consistent and significant difference among VDR genotypes could be observed for the JSN. Calculation of VDR haplotype allele frequencies in ROA cases vs. non-ROA controls showed allele 1 to be overrepresented and allele 3 to be underrepresented in ROA cases as defined by the Kellgren score of ≥ 2 ($\chi^2 = 10.8$, d.f. = 2, $P = 0.005$, for men and women combined) and to a slightly lesser extent as defined by presence of osteophytes ($\chi^2 = 5.91$, d.f. = 2, $P = 0.05$, for men and women combined). This effect was somewhat stronger in men than in women. To estimate the magnitude of the association while adjusting for age and bmi, we performed logistic regression analysis of particular VDR alleles on ROA as assessed by the Kellgren score (Table III). Carriers of VDR allele 2 showed no altered risk for ROA. VDR allele 1 was associated with a 2.27-fold increased risk of ROA while VDR allele 3 was associated with a 1.61-fold de-

Table I. Characteristics of the Population

	Men	Women	All
Number	405	441	846
Age (yr)	68.6±6.9	68.5±6.8	68.5±6.9
bmi (kg/m^2)	25.5±2.9	26.2±3.9	25.9±3.5
Femoral neck bone mineral density (g/cm^2)	0.86±0.14	0.80±0.12	0.83±0.14
Radiographic osteoarthritis at the knee (%)			
Kellgren 2+ (%)	68 (16.7)	111 (25.1)	179 (21.1)
Definite osteophytes (%)	68 (16.7)	114 (26.1)	182 (21.7)
Definite JSN (%; *n*)*	32 (12.4; $n = 259$)	75 (26.3; $n = 285$)	107 (19.7, $n = 544$)

Values are means±standard deviation and percentages (%). *n denotes total number of individuals available for analysis.

Table II. Prevalence by VDR Genotype of ROA and Osteoarthritic Features

	VDR genotype				
	Code	Frequency	Kellgren 2+*	Definite osteophytes*	Definite JSN*
Men	[1,1]	109 (26.9)	21 (19.3)	16 (15.0)	10 (14.1)
	[1,2]	137 (33.8)	28 (20.4)	28 (20.6)	14 (17.1)
	[1,3]	42 (10.4)	8 (19.0)	7 (16.7)	0 (0)
	[2,2]	74 (18.3)	9 (12.2)	15 (20.3)	4 (8.7)
	[2,3]	39 (9.6)	2 (5.1)	2 (5.1)	2 (6.7)
	[3,3]	4 (1.0)	0 (0)	0 (0)	2 (66.7)
	Total	405 (100)	68 (16.7)	68 (16.7)	32 (12.4)
χ^2		—	7.7	6.9	15.1
P		—	0.18	0.23	0.01
Women	[1,1]	119 (27.0)	31 (26.1)	30 (25.6)	19 (25.0)
	[1,2]	163 (37.0)	48 (29.4)	50 (31.1)	28 (25.9)
	[1,3]	43 (9.8)	12 (25.6)	12 (27.9)	7 (23.3)
	[2,2]	82 (18.6)	17 (20.7)	18 (22.2)	16 (30.2)
	[2,3]	31 (7.0)	3 (9.7)	4 (12.9)	5 (31.3)
	[3,3]	3 (0.6)	0 (0)	0 (0)	0 (0)
	Total	441 (100)	111 (25.2)	114 (26.1)	75 (26.4)
χ^2		—	7.6	6.6	1.2
P		—	0.18	0.25	0.94
All	[1,1]	228 (27.0)	52 (22.8)	46 (20.5)	29 (19.7)
	[1,2]	300 (35.5)	76 (25.3)	78 (26.3)	42 (22.1)
	[1,3]	85 (10.0)	20 (23.5)	19 (22.4)	7 (12.5)
	[2,2]	156 (18.4)	26 (16.7)	33 (21.3)	20 (20.2)
	[2,3]	70 (8.3)	5 (7.1)	6 (8.6)	7 (15.2)
	[3,3]	7 (0.8)	0 (0)	0 (0)	2 (50.0)
	Total	846 (100)	179 (21.2)	182 (21.7)	107 (19.7)
χ^2		—	15.8	12.9	5.4
P		—	0.007	0.025	0.36

*Expressed as number (%) per VDR genotype group.

Table III. Odds Ratios of VDR Genotype for ROA Stratified by VDR Allele

VDR genotype			OR‡ ROA by Kellgren score	
Allele	Presence*	*n*	Age- and bmi-adjusted	Age-, bmi-, and BMD-adjusted
1	−	232	2.27	2.31
	+	603	[1.46, 3.52]	[1.48, 3.59]
2	−	315	0.92	0.92
	+	520	[0.64, 1.31]	[0.64 1.31]
3	−	675	0.62	0.64
	+	160	[0.38, 1.01]	[0.39, 1.05]

BMD, bone mineral density. *−, Reference group of men and women without the allele; +, carrying at least one copy of the allele. ‡*OR*, Odds ratio; the 95% confidence interval is presented in brackets.

creased risk of ROA, although this failed to reach significance. When the analysis was repeated while adjusting for bone mineral density at the femoral neck, the odds ratios did not essentially change. When individual features of ROA were analyzed, VDR allele 1 was associated with an increased risk for osteophytes but not for JSN (Table IV). Similarly, VDR allele 3 was associated with a decreased risk for osteophytes and the same point estimate was observed for the JSN, although this was not statistically significant. When the analysis was repeated while adjusting for bone density at the femoral neck, the odds ratios did not essentially change.

Discussion

This population-based study of the elderly, in whom osteoarthritis is prevalent and an important cause of disability, demonstrates an association between ROA at the knee and VDR genotype. The association is independent of bone density and is modified by the diagnostic criterion used to assess ROA. The association appeared to be largely explained by the presence of osteophytes rather than JSN when we assessed these features independently of the presence of ROA. The absence of an allele dose effect is suggestive of a dominant effect.

We do not think that the association we found is caused by selection bias for several reasons. First, selection on genotype is unlikely. Second, the overall prevalence of ROA at the knee of the group of subjects analyzed in this study is similar to that seen in a larger sample from the Rotterdam Study (10) and in other studies (20). Third, VDR genotype frequencies in this population show no significant departures from Hardy-Weinberg equilibrium and the genotype frequencies in the group of subjects analyzed here are very similar to those previously observed in this (13) and other populations (18, 19). Furthermore, the association cannot be explained on the basis of age because VDR genotype frequencies were similar in age groups above and below the median age (data not shown). Our findings are supported by the recent observation of an association of VDR genotype with ROA at the knee as demonstrated in an independent study population of women aged 45–64 yr in the United Kingdom (21).

The Kellgren system used to diagnose ROA is a composite score for grading severity of the disease including the assessment of osteophytes and JSN (3, 16). The dissociation between the composite features of ROA we observed when we repeated the analysis for these separate features independently of the presence of ROA involved the associations to be predominantly driven by osteophytes rather then JSN. This observation might be the result of the dominance of osteophytes in

Table IV. Odds Ratios of VDR Genotype for Features of Radiographic Osteoarthritis Stratified by VDR Allele

VDR genotype		ROA feature			
Allele	Presence*	Osteophytes		JSN	
		n	*OR*‡	*n*	*OR*‡
1	−	232	1.63	149	1.03
	+	603	[1.09, 2.45]	386	[0.64, 1.66]
2	−	315	1.19	203	1.20
	+	520	[0.83, 1.70]	332	[0.76, 1.88]
3	−	675	0.59	431	0.59
	+	160	[0.37, 0.96]	104	[0.32, 1.10]

*−, Reference group of men and women without the allele; +, carrying at least one copy of the allele. ‡*OR*, Odds ratio; the 95% confidence interval is presented in brackets.

diagnosing ROA using the Kellgren score (3, 16, 22) and/or the difficulty in accurately scoring JSN using radiography (23). Because measurement of the joint space was available for only 64% of the individuals (544/846) the power of our study was somewhat lower for analyzing JSN. Yet, our results did not essentially change when we repeated the analyses while mutually adjusting for these features and by performing stratified analyses, i.e., in individuals with and without osteophytes or JSN (data not shown). Therefore, the association of VDR genotype with osteophytes appears independent of JSN and we do not think a scoring bias can explain the association with osteophytes.

Our results could implicate the VDR gene itself to be functionally involved in the etiology of osteoarthritis. VDR genotype has been implicated in determining bone density (11, 12). The VDR allele associated with reduced prevalence of osteoarthritis (3 = bAT) is associated with a modestly reduced bone density in the population of which the current study was a part (13). Therefore, the observation of such an association raises the question whether the VDR genotype association with osteoarthritis is mediated by bone density. This would be in line with the suggestion that decreased bone density could lead to less damage of articular cartilage because of differential impact loading (24). However, adjustment for bone density did not change the relative risks we calculated, thereby demonstrating that the association of VDR genotype with ROA we observed is not mediated by bone density. However, our observation that VDR genotype is mostly associated with the presence of osteophytes suggests a possible functional significance of the VDR gene in relation to this particular feature of ROA. Osteophytes represent osseous and cartilaginous neoplastic tissues displaying many of the stages of human bone turnover and remodeling (25, 26). The VDR is also known to be expressed in osteoblasts (27) and in chondrocytes (28), cell types which can be found in the osteophyte (25). In line with the notion of implicating the vitamin D metabolism in ROA is the recent observation that low serum vitamin D levels are associated with progression of knee ROA (29).

With respect to any functional significance of the involvement of the VDR gene, it has to be emphasized that the polymorphisms we analyzed in the VDR gene are anonymous and have an hitherto unknown relationship with VDR expression. Therefore, we cannot exclude that the VDR gene is in linkage disequilibrium with another nearby gene involved in the pathogenesis of ROA. Indeed, a candidate gene is present proximal of the VDR gene: the COL2A1 gene. Because the COL2A1 gene product is abundantly present in cartilage, an important target tissue in osteoarthritis, sequence variation in this gene has been analyzed in relation to ROA. Although the gene has been implicated in ROA (30–32) controversy remains because other reports did not show evidence for COL2A1 as a candidate gene for osteoarthritis (33, 34). Genetic mapping studies in pedigrees have demonstrated very close linkage between the COL2A1 and VDR loci (35) and the physical distance separating the two loci is < 740 kb (36). Although our results might be explained by linkage disequilibrium of VDR with COL2A1 alleles, it is also possible that both loci are involved and contribute to the inverse relationship between osteoarthritis and osteoporosis. Studying the linkage of COL2A1 alleles with VDR alleles will therefore help in clarifying the relationship between VDR, COL2A1, osteoarthritis, and osteoporosis.

In conclusion, we observed an association between VDR genotype and ROA at the knee. The association was largely explained by the presence of osteophytes rather than JSN, which might indicate involvement of the VDR gene in the molecular mechanisms regulating the development of osteophytes. The close proximity of the VDR gene and the COL2A1 gene might contribute, at least in part, to the observed inverse relationship between osteoporosis and osteoarthritis.

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