

## PAPERS

### Influence of vitamin D receptor genotype on bone mineral density in postmenopausal women: a twin study in Britain

T D Spector, R W Keen, N K Arden, N A Morrison, P J Major, T V Nguyen, P J Kelly, J R Baker, P N Sambrook, J S Lanchbury, J A Eisman

#### Abstract

**Objectives**—To investigate the possible association between vitamin D receptor genotype and bone mineral density in a large group of postmenopausal twins.

**Design**—Cross sectional twin study.

**Setting**—Twin population based in Britain.

**Subjects**—95 dizygotic (non-identical) pairs of twins and 87 monozygotic (identical) pairs of twins aged 50-69 years, postmenopausal, and free of diseases affecting bone, recruited from a national register of twins and with a media campaign.

**Main outcome measures**—Bone mineral density measured at the hip, lumbar spine, forearm, and for the whole body by dual energy x ray absorptiometry in relation to differences in the vitamin D receptor genotype.

**Results**—At all sites the values of bone density among dizygotic twins were more similar in those of the same vitamin D receptor genotype than in those of differing genotype, and the values in the former were closer to the correlations seen in monozygotic twins. Women with the genotype that made them at risk of osteoporotic fracture had an adjusted bone mineral density that was significantly lower by SD 0.5 to 0.6 at the hip, lumbar spine, and for the whole body. The results could not be explained by differences in age, weight, years since menopause, or use of hormone replacement therapy.

**Conclusions**—The findings that in postmenopausal women in Britain bone density—particularly at the hip and spine—is genetically linked and specifically associated with the vitamin D receptor genotypes should lead to novel approaches to the prevention and treatment of osteoporosis.

#### Introduction

Osteoporosis is a disease characterised by low bone mineral density that results in an increased susceptibility to fractures, especially of hip and spine. About 50 000 fractures of the hip and 40 000 vertebral fractures occur annually in England and Wales, and in most countries the incidence of such fractures is increasing.<sup>1</sup> Although one in three women aged over 65 suffers an osteoporotic fracture, the widespread use of preventive treatments such as vitamin D, calcium, and oestrogens is impractical. Targeting individuals at greatest risk is clearly more acceptable provided that they can be accurately identified. Bone densitometry currently provides the best method of predicting future fracture but lacks sufficient sensitivity and specificity to justify widespread screening. If genetic factors were shown to have a great influence on osteoporosis then perhaps a more accurate method of targeting high risk individuals could be developed and the variability of response to environmental

factors such as exercise, diet, and drugs could be explained.

Studies of twins have shown higher concordance of measurements of bone mineral density at the femoral neck and spine in monozygotic (identical) twins than in dizygotic (non-identical) twins, suggesting that bone mineral density is genetically regulated. Quantitative genetic analysis showed that up to 80% of the variance in bone mineral density could be attributable to genetic factors.<sup>2,3</sup> Further studies have shown that genetic factors also contribute to the determination of bone formation and resorption,<sup>4,6</sup> which in turn predicts bone mineral density.<sup>4</sup> The vitamin D receptor is a member of the superfamily of steroid receptors that is believed to have a wide ranging role in the regulation of calcium homeostasis in a variety of tissues.<sup>7</sup> A recent study of 49 pairs of female, Australian, dizygotic twins has shown that common variants of the vitamin D receptor gene identified by restriction fragment polymorphism analysis predict bone density with a mean difference between the genotypes of 15% at the lumbar spine and 10% at the femoral neck.<sup>8</sup> The subjects were from a predominantly premenopausal, Australian, white population, and the applicability of these findings to other populations is unclear but of great interest. We examined the relation between the vitamin D receptor gene and bone density in a large group of older, postmenopausal twins from a geographically distinct site, which allowed us to explore environmental and genetic differences.

#### Methods

We recruited 206 pairs of white, female twins (97 monozygotic, 109 dizygotic) aged 50-69 years and not known to have any bone disease from a British volunteer twin register and with a national media campaign. We determined zygosity with a standardised questionnaire and confirmed it with DNA fingerprinting. Bone mineral density was measured at the lumbar spine, hip, forearm, and for the whole body with dual energy x ray absorptiometry (Hologic QDR-2000). Reproducibility, assessed in 10 healthy volunteers, ranged from 0.8% to 1.8% between the skeletal sites.

DNA was obtained from the 97 dizygotic pairs who were concordant for postmenopausal status. One twin among these pairs had an eating disorder, and she and her cotwin were subsequently excluded from analysis owing to a gross difference in body weight (65 kg *v* 142 kg). Genotyping the vitamin D receptor was unsuccessful in one pair, leaving 95 pairs for analysis. Of the 97 pairs of postmenopausal monozygotic twins, 87 were concordant for menopausal status and were used as an indicator of the maximal level of agreement in genetically identical individuals.

DNA was extracted from white cells of dizygotic

Rheumatology  
Department, United  
Medical and Dental  
School, St Thomas's  
Hospital, London SE1 7EH  
T D Spector, consultant  
rheumatologist  
R W Keen, research fellow of  
Arthritis and Rheumatism  
Council  
N K Arden, Wellcome  
research fellow  
P J Major, research assistant  
J R Baker, research nurse

Molecular  
Immunogenetics Units,  
United Medical and Dental  
School  
J S Lanchbury, senior lecturer

Bone and Mineral Research  
Division, Garvan Institute  
of Medical Research,  
Sydney, Australia  
N A Morrison, senior scientist  
T V Nguyen, statistician  
P J Kelly, senior lecturer  
P N Sambrook, associate  
professor  
J A Eisman, professor

Correspondence to:  
Dr Spector.

TABLE I—Characteristics of 190 postmenopausal dizygotic twins and 174 postmenopausal monozygotic twins. Values are means (SD) unless stated otherwise

Variable	Monozygotic twins (n=174)	Total (n=190)	Dizygotic twins		
			Genotype		
			TT (n=75)	Tt (n=78)	tt (n=37)
Age (years)	59.7 (4.6)	58.7 (5.5)	58.9 (5.5)	58.3 (5.8)	59.2 (4.9)
Height (cm)	160.2 (5.7)	162.1 (6.2)	162.0 (5.6)	163.2 (6.5)	160.0 (6.3)
Weight (kg)	62.5 (9.2)	65.1 (11.2)	63.6 (9.1)	67.0 (12.8)	63.8 (11.2)
Body mass index (kg/m <sup>2</sup> )	24.4 (3.4)	24.8 (4.4)	24.3 (3.6)	25.2 (5.1)	25.0 (4.3)
Age at menopause (years)	49.2 (4.4)	48.9 (4.9)	48.9 (3.8)	48.8 (5.6)	48.9 (5.2)
Years since menopause	10.5 (6.4)	9.9 (7.4)	10.0 (6.3)	9.5 (8.3)	10.3 (7.6)
No (%) ever used hormone replacement therapy	65 (37)	85 (45)	38 (51)	35 (45)	12 (32)
Median (interquartile range) duration of use of hormone replacement therapy (months)	24 (6 to 48)	24 (6 to 48)	24 (6 to 49.5)	24 (9 to 51)	12 (2.75 to 38.25)
No (%) ever smoked	66 (38)	99 (52)	41 (55)	40 (51)	18 (49)

TABLE II—Intraclass correlation coefficients (SE) for monozygotic and dizygotic pairs of twins concordant and discordant for vitamin D receptor genotype

Site	Monozygotic twins (n=87)	Dizygotic twins		
		Total (n=95)	Vitamin D receptor genotype	
			Concordant (n=55)	Discordant (n=40)
Lumbar spine	0.74 (0.05)	0.36 (0.09)	0.36 (0.12)	0.33 (0.14)
Femoral neck	0.76 (0.04)	0.45 (0.08)	0.52 (0.10)	0.31 (0.14)
Ward's triangle	0.71 (0.05)	0.38 (0.09)	0.39 (0.11)	0.26 (0.15)
Total body	0.87 (0.02)	0.38 (0.09)	0.46 (0.11)	0.25 (0.15)
Distal radius	0.77 (0.04)	0.41 (0.08)	0.46 (0.11)	0.29 (0.15)

twins and a 740 base pair fragment of the vitamin D receptor gene on chromosome 12 was amplified by the polymerase chain reaction with primers (5'-CAGAGCATGGACAGGGAGCAAG-3') and (5'-GCAACTCCTCATGGCTGAGGTCTC-3'). Using a restriction enzyme (*TaqI*), we obtained different sized restriction fragments which allowed identification of three distinct genotype groups (TT, Tt, and tt). These are virtually identical to the previously reported genotypes obtained with the *BsmI* enzyme.<sup>9</sup> All polymerase chain reaction analysis was performed blind to clinical information by technicians in Sydney and London using cross validation on duplicate samples and validation against Southern blotting techniques.

#### ANALYSIS

We aimed at assessing the overall genetic effects and effects of vitamin D receptor genotype on bone mineral density independent of other known environmental factors. There were two principal analyses: firstly, a comparison of the similarities of measures of bone mineral density in monozygotic and dizygotic twins and, secondly, analysis of the effect of vitamin D receptor genotype on bone mineral density independent of twin pairings. Intraclass correlation coefficients (denoted by *rMZ* or *rDZ*) are a standard measure of resemblance within pairs in twin studies. They are derived from the difference between the interpair variance and the interpair variance divided by the sum of the variances.<sup>10</sup> Greater correlations in monozygotic than dizygotic twins therefore reflect a genetic influence on that trait, and the proportion of the variance of a trait explained by genetic factors (heritability or *h*<sup>2</sup>) can be estimated from twice their difference ( $h^2 = 2(rMZ - rDZ)$ ).<sup>10</sup> Intraclass correlations were calculated for each skeletal site for the monozygotic pairs and for dizygotic pairs—concordant (identical vitamin D receptor genotype) and discordant (differing vitamin D receptor genotype)—derived from analysis of variance with a twin analysis program (TWINAN 90).<sup>11</sup> Multiple regression analysis was used to adjust for the effects on bone mineral density of age, weight, years since menopause, and use of oestrogen. In the second analysis (independent of twin pairings as

pairs of twins are not independent samples) a double entered analysis of covariance model was used to test the significance of genotype that adjusted simultaneously for similarity of twins as well as for age, years since menopause, use and duration of hormone replacement therapy, and weight using the least square method.<sup>12</sup> Differences in environmental factors between the groups were tested with analysis of variance and the likelihood ratio  $\chi^2$  statistic with Bonferroni's adjustment for multiple comparisons.

#### Results

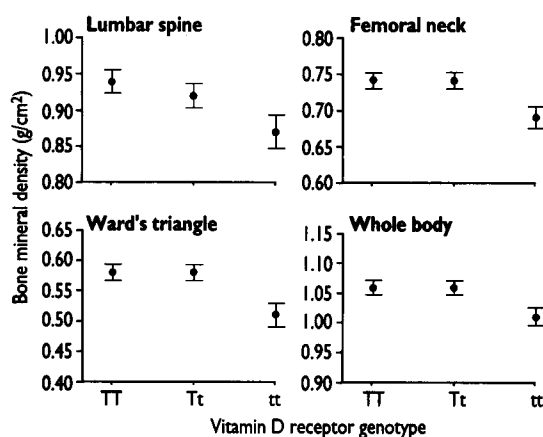
The 95 dizygotic and 87 monozygotic pairs of twins included in the twin analysis were similar in terms of age, height, weight, years since menopause, use and duration of hormone replacement therapy, and smoking history (table I). In the dizygotic pairs the mean age was 58.7 (SD 5.5) years and the average number of years since the menopause was 10.3 (7.4); the corresponding values in the monozygotic pairs were 59.7 (4.6) and 10.5 (6.4). Moreover, the monozygotic and dizygotic pairs had similar means and standard deviations of bone mineral density at all sites, satisfying the general assumptions of twin analysis that the variances should be equal. Analysis of intraclass correlations showed that at all sites the intrapair correlations in monozygotic pairs (*rMZ*) were higher than in dizygotic pairs (*rDZ*) (table II). The bone mineral density for the whole body had the highest heritability (proportion of variance in bone mineral density explained by genetic factors) ( $h^2 = 0.97$ ), followed by the bone mineral densities at the lumbar spine ( $h^2 = 0.76$ ), distal radius ( $h^2 = 0.73$ ), Ward's triangle ( $h^2 = 0.68$ ), and femoral neck ( $h^2 = 0.63$ ).

When dizygotic pairs were classified into vitamin D receptor genotypes the correlation in bone mineral density between concordant pairs (those with identical genotype) was higher than the correlation between the discordant pairs (those with differing genotype) at all sites. At these sites the correlation values seen in the concordant non-identical twins were more similar to those seen in identical twins (table II).

The frequency of the different forms of the vitamin D receptor genotype were 19.5%, 41.0%, and 39.5% for the tt, Tt, and TT groups respectively. Although small variations occurred between the different genotype groups, no significant differences occurred in age, height, weight, body mass index, age at menopause, and smoking status. The heterozygous group, Tt, had a significantly higher weight than the other groups (table I). When the dizygotic twins were treated as individuals there was an overall significant effect of genotype on bone mineral density at all sites except the distal radius, with the tt group having significantly the lowest density at all sites (table III).

The magnitude of this difference, and therefore the

effect of the genotype, may be expressed relative to the standard deviation at each site. As bone mineral density is known to be affected by age, weight, years since menopause, and use of hormone replacement therapy, it was necessary to adjust for these factors before assessing the effect of genotype with analysis of variance. The magnitude of the effect varied little between sites: whole body, SD 0.55 ( $P=0.007$ ); lumbar spine, SD 0.60 ( $P=0.009$ ); Ward's triangle, SD 0.60 ( $P=0.03$ ); and femoral neck, SD 0.53 ( $P=0.02$ , equivalent to a difference of 7-10% (figure). There was no significant effect at the distal radius ( $P=0.13$ ), although the difference was similar (SD 0.48).



Mean (SE) bone mineral density at lumbar spine, femoral neck, Ward's triangle, and for whole body in 190 dizygotic individuals after adjustment for age, weight, duration of menopause, and use and duration of hormone replacement therapy

## Discussion

This study confirms the important link between the vitamin D receptor gene and bone mineral density seen previously in a group of predominantly premenopausal, Australian twins,<sup>9</sup> and extends the relation to postmenopausal women and bone mineral density of the whole body. We have also confirmed the strong genetic component in the determination of bone mineral density at the hip, spine, forearm, and for the first time for the whole body. Monozygotic twins are genetically identical, whereas dizygotic twins are no more alike than siblings, sharing on average 50% of their segregating genes. If a certain gene contributes to an observed genetic effect then dizygotic concordant twins for this gene should be more similar than discordant twins and similar to monozygotic twins. Our data show that correlations between monozygotic pairs were twice as high as between dizygotic pairs, showing that bone mineral density is under strong

genetic regulation. We then showed that in dizygotic twins bone mineral density was more similar in those concordant for the genotype than in discordant twins, and the correlations approached those seen in the monozygotic twins. This suggests that the vitamin D receptor gene contributes to the genetic regulation of bone mineral density. When twins were treated as individuals rather than in pairs a clear relation was seen in the monozygotic women between the vitamin D receptor genotype and bone mineral density at all sites. The extent of the contribution of the genotype was assessed in terms of the standard deviation of differences in bone mineral density between the homozygous genotypes, tt and TT. This ranged from 0.5 to 0.6 and could not be explained on the basis of any other of the measured risk factors at the spine, hip, and for the whole body.

Although our measurement techniques of dual x ray absorptiometry are more precise and reproducible than methods used in previous studies, the intraclass correlations for the monozygotic twins of 0.74 for the lumbar spine and 0.76 for the femoral neck are slightly lower than reported in twin studies using younger subjects.<sup>23</sup> This suggests that with increasing age, shared environmental factors have an increasing influence relative to genetic effects. An additional reason for the lower result at the lumbar spine might be the increased prevalence of spinal osteophytes in older women, which can alter measurements of bone mineral density by up to 25%.<sup>13</sup> Our study is the first to report the strong heritability of the measurement of bone mineral density for the whole body as well as the strong influence of the vitamin D receptor genotype. Confirmation of the effect in dizygotic twins when treated as individuals, importantly shows the generalisability of the results to the wider population, as this group includes women with a wide range of weights, use of hormone replacement therapy, smoking habits, and duration of menopause. The magnitude of the effect of the vitamin D receptor genotype was, however, somewhat reduced when age, weight, and hormone replacement therapy were accounted for, supporting the importance of environmental factors in determination of bone mineral density.

Our findings contrast, however, with those of Hustmyer *et al*,<sup>9</sup> who recently found no relation between different vitamin D receptor genotypes (including *Taq1*) and bone mineral density at the lumbar spine, femoral neck, and forearm. Study design and analytical methods were similar in both studies, and, although we used polymerase chain reaction to identify the genotypes, the two methods have now been cross validated. Our study examined 95 postmenopausal dizygotic pairs with a narrow age range, whereas Hustmyer *et al* examined 39 pairs of women (mean age 43 (SD 11.7) years), of whom only nine dizygotic pairs were concordant for postmenopausal status and use of oestrogen. Frequencies of genotypes were similar in both studies, reflecting a common ancestral background and suggesting that racial differences alone are unlikely to account for the variation in results. If real differences do exist then environmental factors specific to particular populations, such as exposure to vitamin D at an early age, may modify the genetic response and account for conflicting findings between studies.

## CONCLUSION

In conclusion, this study shows a clear association between different vitamin D receptor genotypes and bone mineral density at lumbar spine, hip, and for the whole body in a group of postmenopausal white twins. The importance of this genetic marker lies in its potential role in identifying individual women at increased risk of fracture before menopause and in

TABLE III—Mean (SE) bone mineral density (g/cm<sup>2</sup>) for each vitamin D receptor genotype in 190 dizygotic twins

Site	Genotype			Overall P value
	TT (n=75)	Tt (n=78)	tt (n=37)	
Lumbar spine (first to fourth lumbar vertebrae):				
Crude	0.93 (0.02)	0.94 (0.02)	0.84 (0.03)	0.033
Adjusted	0.94 (0.02)**	0.92 (0.02)	0.87 (0.02)	
Femoral neck:				
Crude	0.73 (0.01)	0.75 (0.01)	0.68 (0.02)	0.034
Adjusted	0.74 (0.01)*	0.74 (0.01)*	0.69 (0.02)	
Ward's triangle:				
Crude	0.58 (0.02)	0.58 (0.02)	0.49 (0.02)	0.008
Adjusted	0.58 (0.01)***	0.58 (0.01)**	0.51 (0.02)	
Total body:				
Crude	1.06 (0.01)	1.07 (0.01)	0.99 (0.02)	0.007
Adjusted	1.06 (0.01)**	1.06 (0.01)***	1.01 (0.02)	
Distal radius:				
Crude	0.43 (0.01)	0.43 (0.01)	0.40 (0.01)	0.133
Adjusted	0.55 (0.01)	0.55 (0.01)	0.53 (0.01)	

Values were adjusted for similarity of twins, age, weight, duration of menopause, and use of hormone replacement therapy with analysis of covariance.

\* $P < 0.05$  v tt; \*\* $P < 0.01$  v tt; \*\*\* $P < 0.005$  v tt.

## Key messages

- Osteoporosis is a disease characterised by low bone mineral density, 80% of which is under genetic control
- Vitamin D has an important role in the metabolism of calcium and bone, mediated through its receptor
- Common variants of the vitamin D receptor gene are responsible for 7-10% of the difference in bone density between women after the menopause
- This genetic marker is important because of its potential role in identifying individual women at increased risk of fracture before menopause and in selecting optimal treatment

selecting optimal treatment based on the understanding of pathophysiological mechanisms. Because of discrepancies between population groups, further studies are needed, with larger sample sizes that include a range of ages in both men and women. The demonstration of the effect of these common vitamin D receptor genotypes on bone mineral density in a second, geographically distinct population of older and postmenopausal women opens the way to a wide range of studies to provide novel approaches to the prevention and treatment of osteoporosis.

This study was funded by grants from the Wellcome Trust, the Arthritis and Rheumatism Council (ARC), the Special Trustees of St Thomas's Hospital, National Health and Medical Research Council of Australia, and National Institutes of Health grant AR 41409. RWK is an ARC clinical

research fellow, and NKA a National Osteoporosis Society research fellow. We thank Kathleen Baan, Christine O'Gara, Maxine Daniels, Gareth Griffiths, Alison MacDonald, and Christel Manzi for help in scanning, recruitment of twins, and data entry; Gabriela Surdulescu for DNA extraction; Charles Slemenda for advice; and Dr Bryan Sykes, John Loughlin, and Catherine Irven of Oxford for the DNA fingerprinting. Lastly we thank the twins themselves for their help.

- 1 Spector TD, Cooper C, Fenton Lewis A. Trends in admissions for hip fracture in England and Wales, 1968-85. *BMJ* 1990;300:1173-4.
- 2 Smith DA, Nance WE, Won Kan K, Christian JC, Johnston CC Jr. Genetic factors in determining bone mass. *J Clin Invest* 1973;52:2800-8.
- 3 Pocock NA, Eisman JA, Hopper JL, Yeates MG, Sambrook PN, Eberl S. Genetic determinants of bone mass in adults: a twin study. *J Clin Invest* 1987;80:706-10.
- 4 Kelly PJ, Hopper JL, Macaskill GT, Pocock NA, Sambrook PN, Eisman JA. Genetic factors in bone turnover. *J Clin Endocrinol Metab* 1991;72:808-13.
- 5 Tokita A, Kelly PJ, Nguyen TV, Leslie AL, Morrison NA, Risteli L, et al. Genetic influences on type I collagen synthesis and degradation: further evidence for genetic regulation of bone turnover. *J Clin Endocrinol Metab* 1994;78:1461-6.
- 6 Garnero P, Arden NK, Delmas PD, Spector TD. Genetic effects in bone turnover. *J Bone Miner Res* 1994;9:S389.
- 7 Farrow S. Allelic variation and the vitamin D receptor. *Lancet* 1994;343:1242.
- 8 Morrison NA, Qi JC, Tokita A, Kelly PJ, Crofts L, Nguyen TV, et al. Prediction of bone density from vitamin D receptor alleles. *Nature* 1994;367:284-7.
- 9 Hustmyer FG, Peacock M, Hui S, Johnston CC, Christian J. Bone mineral density in relation to polymorphism at the vitamin D receptor gene locus. *J Clin Invest* 1994;94:2130-4.
- 10 Falconer DS. *An introduction to quantitative genetics*. London: Longman 1989:163-86.
- 11 Williams CJ, Christian JC, Norton JA Jr. TWINAN90: a FORTRAN program for conducting ANOVA-based and likelihood-based analyses of twin data. *Comput Methods Programs Biomed* 1992;38:167-76.
- 12 DeFries JC, Fulker DW. Multiple regression analysis of twins data. *Behav Genet* 1985;467-73.
- 13 Masud T, Langley S, Wiltshire P, Doyle DV, Spector TD. Effects of spinal osteophytosis on bone mineral density measurements in vertebral osteoporosis. *BMJ* 1993;307:172-3.

(Accepted 30 March 1995)