

Short Report

Polymorphisms of the Vitamin D Receptor Gene Do Not Predict Quantitative Ultrasound of the Calcaneus or Hip Axis Length

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Abstract. Quantitative ultrasound of the calcaneus and hip axis length are independent predictors of hip fracture and have a major genetic component. Polymorphisms of the vitamin D receptor gene (VDR) have been associated with variations in bone density in a number of studies. The aim of this study was to examine the role of VDR on other parameters associated with the risk of fracture. One hundred and eighty-nine pairs of healthy female dizygous twins were genotyped and had calcaneal ultrasound (broadband ultrasound attenuation and velocity of sound) and hip axis length measurements performed. Twin analysis using intraclass correlation coefficients and intrapair differences failed to find an association between the VDR polymorphisms and hip axis length or calcaneal ultrasound. Analysing the twins as a population, irrespective of twinning, also failed to find any association. The search for alternative genes influencing bone fragility should continue as a research priority.

Keywords: Broadband ultrasound attenuation; Hip axis length; Osteoporosis; Twins; Velocity of sound; Vitamin D receptor gene

Introduction

Bone mineral density (BMD) is the main determinant of bone strength and fracture risk. Several studies have shown that BMD has a strong genetic component, with

heritability estimates of 0.5–0.9 [1,2]. One study suggested that up to 75% of the genetic component of BMD can be attributed to polymorphisms of the vitamin D receptor gene (VDR) [3]; however, there is continuing debate as to the exact size and generalizability of the VDR effect [4].

It has been proposed that quantitative ultrasound of the calcaneus measures aspects of bone quality not detected by conventional bone densitometry. There is support for this hypothesis from studies showing that less than 40% of the variance in broadband ultrasound attenuation (BUA) and velocity of sound (VOS) can be explained by BMD [5] measured by dual-energy X-ray absorptiometry (DXA) scanning and that both BUA and VOS can predict fractures independently of their association with BMD [6,7]. BUA and VOS have recently been shown to have a major genetic component, with heritability estimates of 0.53 and 0.61 respectively [8]; however, no published data exist on the VDR gene and ultrasound of the calcaneus.

Hip axis length (HAL), the distance from the greater trochanter through the femoral neck axis to the inner rim of the acetabulum, is an independent predictor of hip fracture [9] and has a major genetic component with a heritability estimate of 0.62 [8]. A preliminary report of a twin study has suggested that femoral neck axis length (FNAL), the distance from the greater trochanter through the femoral neck axis to the edge of the femoral head, is associated with polymorphisms of the VDR [10].

The aim of this study was to examine whether polymorphisms of the VDR are associated with BUA, VOS or HAL in a large population of postmenopausal female twins in which an association between BMD and polymorphisms of the VDR has previously been demonstrated.

Subjects and Methods

One hundred and eighty-nine pairs of female twins aged 50–70 years, 108 monozygous (MZ) and 81 dizygous (DZ), were recruited from two sources: 46% from a twin register at the Institute of Psychiatry, London, where they were initially asked to participate in research into an unspecified range of fields, the other 54% via a national media campaign asking for female twins to take part in a research project on bone and joint problems.

The zygosity of the twins was determined using a validated questionnaire and confirmed using multiplex DNA fingerprinting. All subjects completed a general osteoporosis risk factor questionnaire and those with serious medical illnesses affecting mobility or bone (2 cases of cancer, 1 multiple sclerosis and 1 morbid obesity) were excluded from the study.

BMD of the femoral neck was measured using a Hologic QDR-2000 DXA scanner which produces an automated measurement of HAL. The automated measurement for HAL is calculated using a fan beam and the value obtained will be slightly greater than the true HAL due to the magnification error. Twenty patients underwent duplicate scans, getting off and on the scanner table in between scans, to assess reproducibility. The reproducibility, calculated as a coefficient of variation, was 1.19%. Ultrasound of the calcaneus was measured using a McCue Cuba Clinical scanner. The machine produced two output variables – BUA and VOS – and has a coefficient of variation, assessed by duplicate readings on 30 subjects, of 2.5% and 0.44% respectively.

DNA was extracted from the white cells of DZ pairs and a 740 base pair fragment of the VDR gene was amplified by the polymerase chain reaction. The product was digested with the *TaqI* restriction enzyme allowing identification of three genotypes (*TT*, *Tt*, *tt*). Genotypic polymorphisms were defined as *TT* (absence of restriction site of both alleles), *tt* (presence of restriction site at both alleles) or *Tt* (heterozygote).

To estimate the genetic component of ultrasound measurements and HAL intraclass correlation coefficients were calculated for MZ and DZ twins using the TWINAN90 software package [11]. Intraclass correlations are a standard measure of the similarity within twin pairs and are defined as $r_{MZ} = (AMS - WMS) / (AMS + WMS)$, where AMS is the among mean squares and WMS the within mean squares for MZ twins. The intraclass correlation coefficients for the MZ twins reflects the maximum proportion of variance that can be attributed to genetic factors for any given trait. Only DZ pairs were used to assess the effects of the VDR genotypes; DZ twins were divided into those concordant and discordant for the *TaqI* polymorphisms and two separate analyses performed on the two groups: intraclass correlation coefficients as defined above and intrapair differences. Both analyses were performed on the raw data and also on the residuals obtained after adjusting for potential confounding variables using multiple linear regression. Finally the data were ana-

lysed as population data, irrespective of twin status, using analysis of covariance to test the significance of genotype after adjusting for confounding variables. As there are significant within-pair correlations for all three parameters, data from both members of a pair cannot be included and treated as independent values in the analysis; we therefore randomly selected one member of each MZ and DZ pair for this analysis. With the exception of intraclass correlation coefficients, all analyses were performed using the statistical package for social sciences (SPSS).

Results

There was no significant difference in age, height, weight, duration of menopause or hormone replacement therapy (HRT) usage between the MZ ($n=108$ pairs) and DZ twins ($n=81$ pairs). When the DZ twins were divided into those concordant ($n=46$ pairs) and discordant ($n=29$ pairs) for VDR genotype, they were well matched in terms of age, weight, height, menopause duration, smoking and alcohol use (Table 1). The frequency of the different VDR genotypes was 38.7%, 41.2% and 20.1% for the *TT*, *Tt* and *tt* groups respectively. BMD correlated strongly with BUA ($r=0.59$, $p<0.001$), less strongly with VOS ($r=0.33$, $p<0.001$) and not significantly with HAL ($r=0.06$, $p=0.23$).

Table 1. Baseline characteristics of the dizygous twins

| | Concordant VDR ($n=92$) | Discordant VDR ($n=58$) | <i>p</i> value |
|----------------------------|------------------------------|------------------------------|----------------|
| Age (years) | 60.1 (5.1) | 58.8 (5.5) | 0.15 |
| Weight (kg) | 64.7 (12.0) | 64.3 (8.6) | 0.85 |
| Height (cm) | 162.3 (6.8) | 161.3 (5.5) | 0.31 |
| Menopause duration (years) | 11.8 (6.9) | 10.3 (6.3) | 0.20 |
| HRT use (%) | 38 (41) | 25 (43) | 0.83 |
| Smoking (%) | 45 (49) | 32 (55) | 0.61 |
| Alcohol (%) | 58 (63) | 31 (53) | 0.46 |

Results are presented as means (1 SD), or as number (%) use for HRT, smoking and alcohol.

The results of the adjusted intraclass correlation coefficients are shown in Fig. 1; it can be seen that the r_{MZ} is significantly greater than the r_{DZ} , confirming a major genetic component for all three parameters [8]. The intraclass correlation coefficients for VDR-concordant DZ twins were no greater than those for the VDR-discordant twins for any of the parameters examined. The results of this analysis before adjustments for confounders were essentially unchanged. The intrapair differences were non-significantly greater for the concordant than the discordant twins for: HAL, 0.61 (0.06) vs 0.55 (0.07), $p=0.77$; BUA, 16.9 (1.63) vs 15.6 (1.95), $p=0.66$; and VOS, 46.0 (4.62) vs 40.1 (6.0), $p=0.32$.

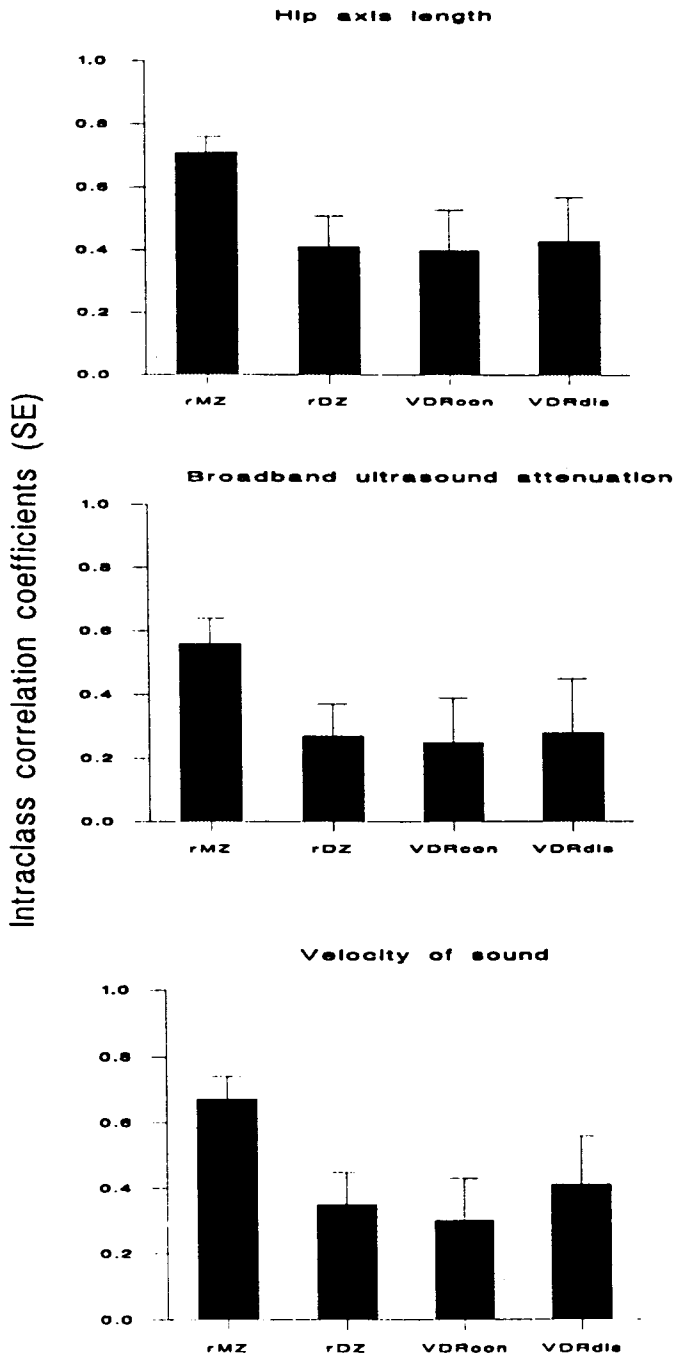


Fig. 1. Intraclass correlation coefficients for monozygous twins (rMZ), all dizygous twins (rDZ), dizygous twins concordant for VDR genotype (VDRcon) and dizygous twins discordant for VDR genotype (VDRdis) after adjustment for age, weight, height and HRT duration.

Adjustments for age, height, weight and HRT duration did not significantly affect the results: HAL, 0.54 (0.06) vs 0.52 (0.07), $p = 0.88$; BUA, 17.1 (1.55) vs 12.9 (2.14), $p = 0.11$; and VOS, 46.7 (4.77) vs 38.2 (5.39), $p = 0.23$. Table 2 shows the results of the population analysis, which also demonstrates no significant difference between genotypes for any of the parameters measured.

Discussion

Although under strong genetic control, HAL, BUA and VOS do not appear to be influenced by VDR genotype. If the VDR had an important genetic influence, the concordant DZ twins would be more similar for the phenotypic trait in question than the discordant DZ twins. This would result in the intraclass correlation coefficients for the concordant twins being greater than those for the discordant twins and approaching the value for the MZ twins; this was clearly not the case in this study. Moreover the intrapair differences for DZ concordant genes would be expected to be smaller than those of the discordant twins, which again was not observed. Analysis of the data irrespective of twin status also failed to show any significant association between VDR and any of the three parameters. This large twin study has adequate power to detect major gene effects on the trait in question, but has limited power to detect minor genes which explain less than 10% of the variance attributed to genetic factors. Although we cannot, therefore, rule out a small effect of the VDR on these parameters, there was no suggestion of this from our data.

We have previously estimated that approximately 60% of the population variance in calcaneal ultrasound is explained by genetic factors; however, these results demonstrate that it is not associated with the VDR. This is a particularly important finding as we have previously shown that the VDR is associated with BMD in the same group of twins. This would suggest that the VDR exerts its effects on BMD via control of bone turnover or mineralization rather than the other aspects of bone structure measured by BUA and VOS. The exact nature of the structural properties of bone measured by calcaneal ultrasound is still uncertain, but there is emerging evidence that BUA measures aspects of bone microarchitecture including trabecular spacing, orientation and connectivity, whereas VOS is measuring bone elasticity as well as density [12].

We found no association between HAL and VDR, which contradicts the provisional results of Hayes et al. [10] reported in abstract form. There are several possible reasons for this discrepancy. We measured HAL which includes both the FNAL studied by Hayes et al. and the acetabular width. Assuming no association with polymorphisms of the VDR, the inclusion of acetabular width in our measurement of HAL could mask an association between FNAL and the VDR. This point warrants further investigation, as it is the acetabular component of HAL which has been reported to have the strongest association with hip fracture risk [13] and may therefore be of greater clinical importance. We cannot exclude that the association is population specific or that important environmental-genetic interactions exist. Further studies of both variables in other populations are required to clarify issue.

In conclusion, calcaneal ultrasound and hip axis length are important predictors of hip fracture and both have a major genetic component. They are not associ-

Table 2. Mean (SE) hip axis length (HAL), calcaneal broadband ultrasound attenuation (BUA) and velocity of sound (VOS) for each VDR genotype in 157 individuals^a

| Site | TT | Tt | tt | p value |
|-------------------|---------------|---------------|---------------|---------|
| <i>Unadjusted</i> | | | | |
| HAL (cm) | 11.37 (0.09) | 11.33 (0.09) | 11.37 (0.14) | 0.87 |
| BUA | 68.68 (2.13) | 73.54 (2.27) | 69.28 (2.65) | 0.24 |
| VOS | 1606.7 (6.23) | 1602.9 (6.65) | 1602.6 (8.47) | 0.89 |
| <i>Adjusted</i> | | | | |
| HAL (cm) | 11.37 (0.09) | 11.34 (0.08) | 11.33 (0.12) | 0.94 |
| BUA | 70.66 (1.94) | 71.52 (1.92) | 71.78 (2.66) | 0.93 |
| VOS | 1608.2 (6.54) | 1600.6 (6.50) | 1604.3 (8.98) | 0.72 |

^aOne individual was randomly selected from each pair. Values were adjusted for age, weight, height and duration of hormone replacement therapy use.

For hip axis length: TT,n=60; Tt,n=67; tt,n=30.

For BUA and VOS: TT,n=53; Tt,n=54; tt,n=28.

ated with polymorphisms of the vitamin D receptor gene and the search for other genes involved in the regulation of bone strength should continue.

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