

Association between vitamin D receptor gene polymorphisms, falls, balance and muscle power: results from two independent studies (APOSS and OPUS)

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Abstract

Summary Fall prevention is a key strategy for reducing osteoporotic fractures. We investigated the association between vitamin D receptor (VDR) polymorphisms and reported falls in postmenopausal women. *Bsm1* polymorphisms were associated with falls, balance and muscle power measurements. These results may explain some of the excess fracture risk associated with VDR in some studies.

Introduction Fall prevention is a key strategy for reducing osteoporotic fractures. It has been suggested that vitamin D supplementation may reduce the incidence of falls by reducing body sway and increasing muscle power. The vitamin D receptor gene is a well-studied candidate gene for osteoporosis.

We investigated the association between VDR polymorphisms and reported falls in postmenopausal women.

Methods Falls data were collected in two separate population cohorts. Five polymorphisms of the VDR gene were analysed (*Cdx-2*, *Fok-1*, *Bsm1*, *Taq1* and *Apal*) in the Aberdeen Prospective Osteoporosis Screening Study (APOSS) cohort. Results found in APOSS were then validated in an independent cohort—the Osteoporosis and Ultrasound (OPUS) study (*Bsm1* and *Fok1* only), where muscle power and balance were also measured.

Results Carriers of the ‘B’ allele (*Bsm1*) showed an increased risk for falls. In APOSS, this was statistically significant for visit 3 multiple falls ($p=0.047$) and for recurrent falls ($p=0.043$). Similar results were found in

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OPUS for visit 1 falls ($p=0.025$) and visit 1 multiple falls ($p=0.015$). *Bsm1* polymorphisms were also associated with balance and muscle power measurements.

Conclusions In conclusion, these results demonstrate an association between the *Bsm1* polymorphism and risk of falling that may explain some of the excess fracture risk associated with VDR in some studies.

Keywords Balance · Falls · Muscle power · Vitamin D receptor gene

Introduction

Osteoporotic fracture is a major cause of morbidity and mortality in elderly men and women. While it is recognised that the major measurable risk factor for fracture in postmenopausal women is low bone mineral density, there is increasing realisation that this does not explain all osteoporotic fractures. Hence, the role of other risk factors, including fall-related aspects, which have an independent effect on fracture occurrence, is receiving increasing interest [1].

The number of falls increases with age [2–5] with approximately 30% of the free-living elderly population falling each year [6]. Up to 90% or more of hip fractures occur following a fall [7].

Risk factors for falls are numerous but include visual impairment, use of specific drugs, such as hypnotics, sedatives and diuretics, chronic conditions including arthritis and Parkinson's disease [3, 8]. Impaired balance and increased body sway have been also shown to be important causes of falls [5, 9, 10], suggesting a role for decreased muscle power. Recent meta-analyses suggest a significant reduction in fall rates of between 12% and 22% when individuals are treated with vitamin D supplementation [11, 12]. The mechanism of action appears to be acting by a reduction in body sway [13] and this, plus an improvement in lower body strength, may reduce the number of falls and hence fractures [14].

Many studies have been performed to date studying the association between candidate gene polymorphisms and bone mineral density (BMD), as a quantitative trait, and fracture risk. There has also been a study suggesting that falls have a hereditary component [15], but despite this, there have been only three studies of which we are aware examining the relationship between gene polymorphisms and falls. A study into vitamin D receptor (VDR) and falls has been previously conducted but in a small group ($n=259$) of very elderly (over 80 years) women. This study found an association between *Bsm1* and falls, within 90 days of assessment, with a reduction in falls for those having the 'b' homozygote, while no association was found for *FokI* [16]. A pharmacogenetic interaction has been noted between PvuII polymorphism of the oestrogen receptor alpha (ESR1) gene

and falls [17], where an association was found for women taking hormone replacement therapy (HRT), but there was no association in non-HRT users. In another study examining apolipoprotein E (APOE) polymorphism, no association with falls was found [18].

The focus of research on the role of VDR polymorphisms has been on bone health, and few studies have examined the associations between VDR and muscle strength [19–21, 16]. Two studies [19, 22] have found that the presence of the b allele of the *Bsm1* polymorphism is associated with increased quadriceps strength, although other studies have found no such association [20, 21]. Examining lower limb power, the presence of the B allele was associated with increased hamstring strength in one study [20], but no such association was found in another [21]. For grip strength, no significant associations with *Bsm1* polymorphism of the VDR gene have been reported [22, 20, 16], although a trend was found for increased grip strength [19] with the 'b' allele. If a relationship did exist between VDR gene polymorphisms and falls, this might provide an efficient means of targeting vitamin D treatment to produce an enhanced reduction on fall and fracture rates.

Given the associations found between VDR gene polymorphisms and muscle strength, our aim was to determine the nature of the relationship between VDR gene polymorphisms and fall risk.

Methods

Aberdeen Prospective Osteoporosis Screening Study cohort

The Aberdeen Prospective Osteoporosis Screening Study (APOSS) population arises from a community-based study, involving women initially aged between 45 and 54 years of age at the baseline visit. The women were chosen at random from a primary care patient register, the Community Health Index, and lived within a 32-km radius of the Osteoporosis Screening Unit, Aberdeen. Between 1990 and 1993 ("baseline"), a letter was sent directly from the screening unit inviting 7,200 potential participants to take part in the screening programme, which consisted primarily of a dual-energy X-ray absorptiometry scan of the hip and spine using a Norland XR-26 and XR-36 Dual-energy X-ray Absorptiometry scanners, and completion of a risk factor questionnaire. Five thousand one hundred nineteen attended for screening.

Between 1997 and 2000 ("visit 2"), the women who had taken part in the study were invited to attend a second screening visit, and 3,883 attended. Falls data were collected using the question "Have you fallen in the last year?" At this time point, those who consented gave a blood sample for DNA analysis. Genotyping of the vitamin D receptor was performed in the Aberdeen bone laboratories

using genomic DNA, extracted using standard methodology from peripheral blood leukocytes and stored in 96-well plates at -20°C . Five polymorphic regions of VDR were genotyped. These were the exon 2 *FokI* polymorphism, which creates an alternative translational start site; the functional polymorphism for the *Cdx-2* transcription factor binding site, which is located within the promoter region (*Cdx-2*); and finally, *BsmI*, *Apal* and *TaqI* in the 3' region of the gene.

The genotyping methodology has been described in detail previously [23]. Briefly, genotypes for both the *Apal* and *TaqI* polymorphisms were obtained by polymerase chain reaction restriction fragment length polymorphism (RFLP) and visualised on ethidium bromide stained agarose gel. *FokI* and *BsmI* genotypes were determined primarily ($> 89\%$) using the 5' nuclease Taqman assay (Applied Biosystems, Foster City, CA, USA) and the remaining samples by RFLP. The concordance rate between the two methods is 98%. The *Cdx-2* SNP was genotyped using the DYEnamic ET dye terminator cycle sequencing kit (Amersham Pharmacia Biotech UK) and analysed on a MEGABACE-1000 automated DNA sequencer (Amersham).

Serum samples were analysed for parathyroid hormone (PTH) by a two-site immunoradiometric assay for the intact 84 amino acid chain of PTH (Nichols Institute Diagnostic, San Juan, Capistrano, USA). The assay has been validated by extensive clinical studies in well-defined populations of various parathyroid disease states and is sensitive to 0.9 pM PTH(1–84). The inter-assay coefficient of variation (CV) was 5.6–6.1%, and the intra-assay variation was 1.8–3.4% (measured in samples of mean 40–266 pM). Vitamin D status was determined by measuring 25(OH)D by high-performance liquid chromatography. Serum samples were deproteinised with acetonitrile and centrifuged. The supernatant was introduced onto a Varian Bond Elut C18 cartridge. Roche provided the internal standard. Pre-assayed aliquots of calf serum were used as internal controls. To provide external control, analysts from different laboratories routinely compared results of prepared samples (DEQAS). Within-batch precision was 7.9–13.5% CV, and between-batch precision was 11.9–12.2% CV across the range of 4–100 ng/ml (10–250 nM). Due to the seasonal nature of 25(OH)D [24], we also noted the month the sample was given to allow adjustment for seasonality. Dietary vitamin D assessment was made using a validated food frequency questionnaire and analysed using a UK database [25], and sunlight exposure was assessed using a sunlight questionnaire.

In 2002 (“visit 3”), 2–5 years after the second visit, a postal risk factor questionnaire was sent, and 3,145 returned a completed form. The questionnaire enquired about the presence of incident fractures and falls using the falls question as outlined above for visit 2. Those who indicated the number of falls in the last year (at visit 3 only) to be greater than one were classed as “multiple fallers”.

Those who responded positively to the falls question at both time points (i.e. visits 2 and 3) were classed as “recurrent fallers”.

Written informed consent was obtained from all participants. The Grampian Research Ethics Committee approved the study.

Osteoporosis and Ultrasound cohort

The Osteoporosis and Ultrasound (OPUS) population was recruited in five centres (Aberdeen, Berlin, Kiel, Sheffield and Paris) in 1999 to 2001 (“baseline”). The population has been described in detail elsewhere [26], but in brief, all subjects were randomly selected in age-stratified bands. A younger postmenopausal group of women aged 20 to 40 years was recruited ($n=100$ from each centre) but was not included in this analysis. For the older postmenopausal group (aged 55 to 80 years) included in this analysis, each centre aimed to recruit 100 subjects in five 5-year age bands. In total, 2,374 older women were included in this analysis. Serum 25(OH)D was measured in a random sub-set ($n=934$) by radioimmunoassay (IDS Ltd, Tyne and Wear, UK). As with the APOSS cohort, we also noted the month the sample was given to allow adjustment for seasonality. Serum PTH ($n=2,269$) was analysed using immunoassay (Elecsys 2010 immunoanalyser; Roche Diagnostics GmbH, Mannheim, Germany), with a coefficient of variation of 1.3%. DNA was extracted from whole blood with a modification of the ‘salt-out’ technique (Nucleon IITM, Scotlab, Paisley, Scotland). Extracted DNA was suspended at a concentration of 200 $\mu\text{g}/\text{ml}$ and stored at -20°C . *BsmI* and *FokI* have been genotyped in 1,970 subjects to date using the 5' nuclease assay (Taqman[®]; Hoffman-LaRoche, Inc., Nutley, NJ, USA). Falls data were collected using the question “Have you fallen in the last year?” with positive respondents then asked to estimate the number of falls they had suffered.

Balance and muscle power were measured. All subjects had grip strength measured using a dynamometer (Takei Grip-D, Takei Scientific, Japan). Measurements were taken twice in both hands and an average noted of the four measurements. The subjects were also asked to get up from a chair (a chair with arms plus a chair without arms). It was noted by the researcher whether the subject was able to get up “easily”, “with some difficulty”, “with difficulty”, “very difficult” or “not possible”. In addition, subjects were asked to walk along a 5-m line marked on the floor without moving off the line. Each subject was asked to walk on the line forwards with normal steps, backwards with normal steps, forwards toe-to-toe and backwards toe-to-toe. The distance at which they stepped off the line was noted (in 10-cm lengths). A ground reaction force plate was used to measure leg force ($n=578$). Each subject was asked to stand up quickly, straightening their knees, and sit down

immediately. Three measurements were performed and the average used. Measurements recorded were power (kg), force per weight (N/kg), total force (kN), speed (m/s) and total power (kW).

A second visit was completed between 2005 and 2007 (“visit2”) where further examinations/questionnaires were completed. Written informed consent was obtained from all participants. The study was approved by the appropriate ethics committee at each participating centre.

Multiple fallers are classified as those reporting greater than one fall in the past year (baseline and visit 2). Recurrent fallers are those who indicated at least one fall at both time points.

Statistical analysis

Analyses were performed using SPSS version 15.0 (SPSS Chicago, IL, USA). Logistic regression was used to determine falls risk by calculating odds ratios (OR) and their associated 95% confidence intervals (95% CI). *t* tests, one-way analysis of variance (ANOVA) and chi-square were also used where appropriate. Chi-square test for Hardy Weinberg equilibrium was performed using Hardy Weinberg Test version 1.10 (Utility Programs for analysis of genetic linkage, J.Ott).

Results

The baseline characteristics of the populations are shown in Table 1. In APOSS, 853 women (22.2%) indicated having sustained a fall in the previous 12 months. In OPUS, where the women are older, 670 of 2,363 (28.4%) indicated a fall at baseline. We categorised the OPUS women according to age group, and the percentage of fallers increased with age

(percentage of fallers by age group: 55–59 years=25%, 60–64 years=28.2%, 65–69 years=28.0%, 70–74 years=28.6% and 75+ years=33.1%). Using chi-square, this was not statistically significant ($\chi^2=6.17$, $p=0.187$); however, when a chi-square test for trend was applied, this was statistically significant ($\chi^2=5.05$, $p=0.025$). Genotype frequencies are shown in Table 2, with chi-square results for deviation from Hardy Weinberg equilibrium. In the *Cdx-2* polymorphism, 64.0% of the APOSS cohort was AA homozygote, 31.6% AG heterozygote and 4.4% GG homozygote. In the *FokI*, 38.4% of the APOSS cohort was FF homozygote, 46.2% Ff heterozygote and 15.4% ff homozygote, this compared with 34.5% FF homozygote, 49.5% Ff heterozygote and 16.0% ff homozygote in the OPUS cohort. In the *BsmI* polymorphism, 33.9% of the APOSS cohort was bb homozygote, 47.3% Bb heterozygote and 18.9% BB homozygote compared with 35.5% bb homozygote, 49.5% Bb heterozygote and 15.3% BB homozygote in the OPUS cohort. In the *Apal* polymorphism, 31.2% of the APOSS cohort was AA homozygote, 49.8% Aa heterozygote, and 19.0% aa homozygote. In the *TaqI* polymorphism, 35.6% of the APOSS cohort was TT homozygotes, 48.2% was Tt heterozygote, and 16.2% was tt homozygote. Table 3 shows the genotype frequencies with regard to faller status. 25(OH)D differed significantly ($p<0.001$) when examining the month of measurement for both APOSS and OPUS cohorts.

Vitamin D/PTH and fall risk

APOSS cohort

We compared 25(OH)D and PTH in the fallers and non-fallers at visit 2 only, since the samples were taken at this

Table 1 Baseline characteristics of both APOSS and OPUS populations

Variable	APOSS		OPUS	
	Mean	Standard deviation	Mean	Standard deviation
Age (years)	54.3	2.3	66.9	7.0
Height (cm)	160.3	6.0	160.4	6.2
Weight (kg)	68.8	12.9	68.7	12.4
Age at menopause	47.1	5.9	48.4	6.0
Post menopausal (%)	90.2		100.0	
Fallers (%)	Baseline = not applicable Visit 2 = 853/3,848 (22.2%) Visit 3 = 487/3,271 (14.9%)		Baseline = 668/2,363 (28.3%) Visit 2 = 586/1,706 (34.6%)	
Multiple fallers (%)	Baseline = not applicable Visit 2 = not applicable Visit 3 = 238/3,255 (7.3%)		Baseline = 239/2,277 (10.5%) Visit 2 = 260/1,706 (15.2%)	
Recurrent fallers (%) i.e. reported fall at both different time points	157/3,120 (5.0%)		240/1,698 (14.1%)	

Table 2 Genotype distribution

Polymorphism	APOSS		OPUS	
Cdx-2				
AA	1,997 (64.0%)	$\chi^2=1.18$;	n/a	
AG	986 (31.6%)	$p=0.277$	n/a	
GG	137 (4.4%)		n/a	
Total	3,120			
FokI				
FF	1,182 (38.4%)	$\chi^2=1.78$;	680 (34.5%)	$\chi^2=1.24$;
Ff	1,423 (46.2%)	$p=0.182$	975 (49.5%)	$p=0.266$
ff	474 (15.4%)		315 (16.0%)	
Total	3,079		1,970	
BsmI				
bb	1,066 (33.9%)	$\chi^2=3.41$;	700 (35.5%)	$\chi^2=1.31$;
Bb	1,488 (47.3%)	$p=0.065$	969 (49.5%)	$p=0.251$
BB	594 (18.9%)		301 (15.3%)	
Total	3,148		1,970	
Apal				
AA	1,010 (31.2%)	$\chi^2=0.331$;	n/a	
Aa	1,609 (49.8%)	$p=0.565$	n/a	
aa	615 (19.0%)		n/a	
Total	3,234			
TaqI				
TT	1,144 (35.6%)	$\chi^2=0.008$;	n/a	
Tt	1,546 (48.2%)	$p=0.931$	n/a	
tt	519 (16.2%)		n/a	
Total	3,209			

visit. We found no significant differences in 25(OH)D ($p=0.570$), but PTH was significantly higher in the group who had fallen (Mean PTH (SD): fallers = 3.55 ng/L (2.44) vs. non-fallers = 3.32 ng/L (2.05); $p=0.041$). It was noted that there was a high SD relative to the mean, suggesting non-normal distribution. However, after ln transformation of these data, the result remained significant ($p=0.012$).

OPUS cohort

No significant differences in 25(OH)D were found between those who fell in previous 12 months compared to those who did not fall in OPUS (mean (SD): fallers=50.1 nmol/L (23.8); non-fallers = 49.8 nmol/L(22.3); $p=0.850$). Similarly no significant differences were found comparing multiple fallers vs. fallen once vs. non-fallers for 25(OH)D (mean (SD): multiple fallers = 48.1 nmol/L (20.5); fallen once = 52.3 nmol/L (26.5); non-fallers = 49.8 nmol/L (22.3); $p=0.336$). Although we found no significant difference in PTH between fallers and non-fallers (mean (SD): fallers = 38.50 ng/L (21.21); non-fallers = 37.16 ng/L (19.80); $p=0.157$), there was a significant difference in PTH for multiple fallers (mean (SD); non-fallers = 37.2 ng/L

(19.8); fallen once = 37.0 ng/L (19.0); multiple fallers = 40.4 ng/L (23.5); p value = 0.040).

APOSS cohort

VDR polymorphism and fall risk

With fallers at either time point using the dominant model, BsmI B carriers showed an increase in falls risk, although this was not significant ($p=0.329$; Table 4).

None of the other gene polymorphisms showed significant associations with falls at either time point in the APOSS cohort (*Cdx-2*: $p=0.752$; *Apa-1*: $p=0.525$; *Taq-1*: $p=0.303$; *Fok-1*: $p=0.566$; Table 4).

In APOSS, we examined multiple fallers at visit 3 and found an increased risk for carriers of the 'B' allele compared to 'b' homozygotes ($p=0.047$; Table 4). This association remained when adjusting for 25(OH)D and month of measurement ($p=0.043$).

None of the other gene polymorphisms showed a significant association with multiple falls at visit 3, although *FokI* did approach significance where 'f' homozygote showed an increase in fall risk compared to 'F' homozygote ($p=0.054$; Table 4).

Recurrent fallers ($n=126$), i.e. those reporting a fall at visits 2 and 3, showed an overall trend for increased risk for carriers of the 'B' allele when compared to non-fallers ($n=2,398$; age, height and weight adjusted OR (95% CI); BB/Bb = 1.527 (1.041–2.298) bb = reference, $p=0.043$). This trend remained after adjustment for 25(OH)D and month of measurement (age, height and weight adjusted OR (95% CI); BB/Bb = 1.523 (1.002–2.316) bb = reference, $p=0.049$).

None of the other gene polymorphisms showed a significant association with recurrent falls (*Cdx-2*: $p=0.366$; *Apa-1*: $p=0.401$; *Taq-1*: $p=0.722$; *Fok-1*: $p=0.632$).

VDR polymorphisms and vitamin D/parathyroid hormone

Serum 25(OH)D was examined in relationship to VDR polymorphisms. No relationship was found for *Cdx2* ($p=0.892$), *BsmI* ($p=0.415$), *FokI* ($p=0.728$), *Apal* ($p=0.956$) or *TaqI* ($p=0.789$). We also examined PTH levels and found only one significant association with *Cdx2* ($p=0.023$), where those with the 'G' homozygote had significantly reduced PTH. No significant associations were found for *BsmI* (0.941), *FokI* ($p=0.937$), *Apal* ($p=0.527$) or *TaqI* ($p=0.776$).

OPUS cohort

VDR polymorphism and fall risk

At baseline, we found that carriers of the 'B' allele were at significantly increased risks for falls ($p=0.025$;

Table 3 Genotype frequencies (%) for fallers and multiple fallers

Polymorphism	APOSS				OPUS					
	Non-fallers ^a	Fallers	Non-fallers ^b	Fallen once	Multiple fallers	Non-fallers	Fallers	Non-fallers	Fallen once	Multiple fallers
<i>Cdx-2</i>										
AA	1,542 (64.1%)	437 (64.5%)	1,390 (64.1%)	114 (66.3%)	125 (65.1%)	n/a	100 (18.1%)	214 (15.2%)	58 (20.8%)	31 (15.5%)
AG	757 (31.5%)	210 (31.0%)	682 (31.4%)	54 (31.4%)	58 (30.2%)	n/a	264 (47.8%)	705 (50.0%)	131 (47.0%)	93 (46.5%)
GG	106 (4.4%)	31 (4.6%)	98 (4.5%)	4 (2.3%)	9 (4.7%)		188 (34.1%)	490 (34.8%)	90 (32.3%)	76 (38.0%)
Chi-square	$p=0.959$		$p=0.732$					$p=0.291$		$p=0.166$
<i>FokI</i>										
FF	912 (38.4%)	257 (38.6%)	825 (38.4%)	67 (40.1%)	64 (34.6%)		100 (18.1%)	215 (15.2%)	58 (20.8%)	31 (15.5%)
Ff	1,102 (46.3%)	305 (45.8%)	987 (45.9%)	74 (44.3%)	83 (44.9%)		264 (47.8%)	705 (50.0%)	131 (47.0%)	93 (46.5%)
ff	364 (15.3%)	104 (15.6%)	337 (15.7%)	26 (15.6%)	38 (20.5%)		188 (34.1%)	490 (34.8%)	90 (32.3%)	76 (38.0%)
Chi-square	$p=0.964$		$p=0.483$							
<i>BsmI</i>										
bb	824 (33.9%)	231 (33.9%)	757 (34.5%)	61 (35.7%)	52 (27.5%)		173 (31.3%)	522 (37.0%)	88 (31.5%)	55 (27.5%)
Bb	1,167 (48.0%)	306 (44.9%)	1,026 (46.7%)	86 (50.3%)	93 (49.2%)		271 (49.1%)	695 (49.3%)	146 (52.3%)	96 (48.0%)
BB	438 (18.0%)	145 (21.3%)	413 (18.8%)	24 (14.0%)	44 (23.3%)		109 (19.6%)	193 (13.7%)	45 (16.1%)	49 (24.5%)
Chi-square	$p=0.129$		$p=0.120$					$p=0.002$		$p=0.001$
<i>ApaI</i>										
AA	781 (31.3%)	214 (30.6%)	708 (31.4%)	48 (26.8%)	62 (31.8%)		173 (31.3%)	522 (37.0%)	88 (31.5%)	55 (27.5%)
Aa	1,239 (49.6%)	355 (50.7%)	1,113 (49.3%)	100 (55.9%)	97 (49.7%)		271 (49.1%)	695 (49.3%)	146 (52.3%)	96 (48.0%)
aa	477 (19.1%)	131 (18.7%)	437 (19.4%)	31 (17.3%)	36 (18.5%)		109 (19.6%)	193 (13.7%)	45 (16.1%)	49 (24.5%)
Chi-square	$p=0.877$		$p=0.560$							
<i>TaqI</i>										
TT	870 (35.1%)	260 (37.4%)	802 (35.7%)	69 (39.2%)	68 (36.0%)		173 (31.3%)	522 (37.0%)	88 (31.5%)	55 (27.5%)
Tt	1,217 (49.2%)	315 (45.3%)	1,078 (48.0%)	89 (50.6%)	84 (44.4%)		271 (49.1%)	695 (49.3%)	146 (52.3%)	96 (48.0%)
tt	389 (15.7%)	121 (17.4%)	365 (16.3%)	18 (10.2%)	37 (19.6%)		109 (19.6%)	193 (13.7%)	45 (16.1%)	49 (24.5%)
Chi-square	$p=0.182$		$p=0.164$							

^a Visit 2 fallers^b Visit 3 fallers, as number of falls not asked at visit 2

Table 4 Logistic regression non-fallers versus ever fallen or multiple fallers for both APOSS and OPUS cohorts. All data adjusted for age, height and weight

Polymorphism	APOSS		OPUS	
	Non-fallers vs ever fallen	Non-fallers vs multiple fallers (visit 3)	Non-fallers vs baseline fallers	Non-fallers vs multiple fallers (baseline)
<i>Cdx-2</i> carrier <i>G</i> vs <i>AA</i>	0.973 (0.815–1.163)	0.940 (0.689–1.282)	Not applicable	Not applicable
<i>FokI</i> <i>F</i> carrier vs <i>ff</i>	0.923 (0.733–1.163)	1.516 (0.992–2.315)	1.026 (0.833–1.263)	0.853 (0.631–1.168)
<i>BsmI</i> <i>B</i> carriers vs <i>bb</i>	1.047 (0.725–1.114)	1.401 (1.005–1.953)	1.272 (1.030–1.571)	1.505 (1.081–2.095)
<i>ApaI</i> <i>A</i> carrier vs <i>aa</i>	1.100 (0.887–1.364)	1.066 (0.731–1.555)	Not applicable	Not applicable
<i>TaqI</i> <i>T</i> carrier vs <i>tt</i>	0.949 (0.756–1.192)	0.802 (0.550–1.170)	Not applicable	Not applicable

Table 4). After further adjustment for 25(OH)D and month of measurement, the association remained significant ($p=0.007$).

In the OPUS cohort, grip strength was measured in 658 women allowing additional adjustment. Carriers of the ‘B’ allele were still significantly at increased risk after further adjustment for grip strength (OR (95% CI); BB/Bb = 1.623 (1.112–2.368); bb = reference, $p=0.012$).

No significant association was found for *FokI* polymorphism ($p=0.349$) and baseline falls.

At baseline, multiple fallers (i.e. those women who reported more than one fall in the previous year), carriers of the ‘B’ allele, showed an even stronger association when adjusted for age, height and weight compared to non-fallers ($p=0.015$; Table 4). The significance of the relationship was lost after adjustment for 25(OH)D plus month of measurement ($p=0.083$) and, additionally, grip strength ($p=0.104$).

No significant associations were found for both *BsmI* and *FokI* with regard to visit 2 falls, visit 2 multiple falls nor with recurrent falls (data not shown).

VDR polymorphisms and vitamin D/parathyroid hormone

In the OPUS cohort, using ANOVA, we compared 25(OH)D levels across the *BsmI* polymorphisms. The bb homozygote was found to have higher levels of 25 (OH)D, although this failed to reach significance ($p=0.09$). Similarly, we compared PTH levels across *BsmI* polymorphisms, and this showed a significant difference across genotypes ($p=0.043$) with bb showing the lowest PTH level. No differences were found for either 25(OH)D or PTH using the *FokI* polymorphisms (p values = 0.387 and 0.827, respectively).

VDR polymorphisms and balance/muscle power

In the rise from the chair, comparing test subjects who found it “easy” to the remaining subjects, there was a significant difference between the *BsmI* polymorphisms, with more subjects finding it “easy” to rise from a chair with arms ($p=0.023$). Similarly, in the rise from a chair without arms, there was a difference between the *BsmI* polymorphisms, although this failed to reach significance ($p=0.079$). Differences between the *BsmI* polymorphisms approached significance for both maximum power ($p=0.080$) and the 5-m walk/balance test (forward, normal steps; $p=0.096$).

We examined bb homozygotes vs. carriers of the B allele since bb seems to be protective for falls. For the leg force measurements, significant differences were found for maximum power (mean (SD): bb=0.48 kW (0.15), Bb/BB = 0.45 kW (0.14); $p=0.044$), and differences approached significance ($p=0.098$) for maximum force (mean (SD): bb = 0.82 kN (0.12), Bb/BB = 0.80 kN (0.13)). There was a difference approaching significance for grip strength (mean (SD); bb = 19.5 kg (5.8), Bb/BB = 18.9 kg (5.6); $p=0.06$). None of the 5-m walk/balance test measurements showed significant differences between genotypes (p values from 0.302 to 0.703). For rising from chair, there were significant differences in both rise from chair with arms (% “easy”; bb = 82.3%, Bb/BB = 77.1%; $p=0.008$) and rise from chair without arms (% “easy”; bb = 87.4%, Bb/BB = 83.7%; $p=0.030$; Table 5).

No associations were found for *FokI* and balance/muscle power measurements.

Table 5 OPUS *BsmI* muscle power and balance

	bb	Carriers B	p value
Max power (kW) mean \pm SD	0.48 \pm 0.15	0.45 \pm 0.14	0.044
Max force (kN) mean \pm SD	0.82 \pm 0.12	0.80 \pm 0.13	0.10
Grip strength (kg) mean \pm SD	19.5 \pm 5.8	18.9 \pm 5.6	0.06
Rising from chair with arms (% easy)	82.3	71.1	<0.01
Rising from chair without arms (% easy)	87.4	83.7	0.03

Vitamin D/PTH and fall risk

No significant differences in 25(OH)D were found between those who fell in previous 12 months compared to those who did not fall in OPUS (mean (SD): fallers = 50.12 nmol/L (23.77); non-fallers = 49.81 nmol/L(22.31); $p=0.850$). Similarly, no significant differences were found comparing multiple fallers vs. fallen once vs. non-fallers for 25(OH)D (mean (SD): multiple fallers = 48.08 nmol/L (20.45); fallen once = 52.29 nmol/L (26.50); non-fallers = 49.81 nmol/L (22.31); $p=0.336$). Although we found no significant difference in PTH between fallers and non-fallers (mean (SD): fallers = 38.50 ng/L (21.21); non-fallers = 37.16 ng/L (19.80); $p=0.157$), there was a significant difference in PTH for multiple fallers (mean (SD); non-fallers = 37.2 ng/L (19.8); fallen once = 37.0 ng/L (19.0); multiple fallers = 40.4 ng/L (23.5); p value = 0.040).

Bsm1 gene dose effect

Although a monotonic increase was observed in both cohorts, no significant gene dose effect was found in either cohort. In the APOSS cohort, the percentage of women reporting a fall at either time point was 29.8%, 30.0% and 32.3%, respectively, for genotypes bb, Bb and BB (χ^2 $p=0.60$; test for trend $p=0.39$). Similarly in the OPUS cohort, the percentage of women reporting a fall at either time point was 46.5%, 48.5% and 52.5%, respectively, for bb, Bb and BB (χ^2 $p=0.33$; test for trend $p=0.16$).

Discussion

Our data show an association between falls and polymorphisms in *Bsm1* with carriers of the B allele at higher risk of falls in both cohorts in postmenopausal women. To our knowledge, only one previous study has examined this association [16] between *Bsm1* or *Fok1* and falls. They found a similar association, with the ‘B’ homozygote showing an increased risk of falls for *Bsm1* and no association for *Fok1*. However, their study was in a very elderly group of women (mean age = 85 years), and the sample size was much smaller than our current study ($n=259$). Our current study also shows that the association is independent of serum 25-hydroxy-vitamin D. The effects in our current study were stronger when multiple fallers were compared to non-fallers. The ‘b’ homozygote of *Bsm1* was associated with higher serum 25-hydroxy-vitamin D, lower parathyroid hormone and stronger measures of muscle force.

In the APOSS cohort, approximately one fifth reported falling, while this may seem quite high for a relatively

young population (mean age 54 years) this is in accordance with fall rates reported elsewhere[27, 28].

In the OPUS cohort, *Bsm1* was associated with falls risk at baseline but was no longer significantly associated at visit2. This may in part be due to loss to follow-up of the frailer members of the cohort. The OPUS cohort are an elderly cohort (mean age 66.9 years). Analysis of a simple general health question revealed that those who did not attend their follow-up visit were more likely to report poorer health, 14.4% versus 10.6% for those who attended.

VDR is a nuclear hormone receptor which was the first to be implicated in osteoporosis [29, 30] when it was noted to relate to BMD. The VDR receptor gene has been targeted in the research of the genetic determinants influencing bone status because it regulates bone homeostasis through the vitamin D endocrine system [31]. The large number of polymorphic sites and a profusion of small and underpowered studies have shown conflicting results for the effects of the polymorphisms on BMD and fractures. Recent examination of all five polymorphisms and their haplotypes in our own population of over 3,000 women failed to show a relationship with BMD or fracture, although with only 248 validated fractures, the latter analysis was underpowered [23]. Indeed, recent studies [32, 33] have shown a relationship between VDR and risk of fracture, which was independent of BMD, but the mechanism by which VDR influences fracture risk was not determined. Also, in a genome-wide search of the top loci associated with BMD, VDR was only found when the search was expanded to the top 1,000 loci [34]. A recent meta-analysis [35] involving over 26,000 participants again showed no significant relationship between any of the five polymorphisms, and BMD but did show a weak relationship between the *Cdx-2* polymorphism and vertebral fractures. Assuming this association to be correct, this would indicate an alternative mechanism other than BMD, and one option would be relationships between VDR polymorphisms and falls.

There are a number of limitations associated with this study. Falls data were collected using the question “Have you fallen in the last year?” This has not been validated

In addition, multiple testing has been carried out, although this is justified since the outcomes are correlated. The strength of this study is that this is a large study examining two populations. We have also various measures of muscle power, balance and a measure of 25(OH)D.

Although we found a relationship between these polymorphisms and falls, it is possible that it is a gene located in this area of the chromosome that has yet to be characterised and not the VDR gene that is related to falls. Further work is required to confirm these findings and to elucidate the mechanism of the effect.

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Conflicts of interest None.

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