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Vitamin D receptor (VDR) gene polymorphism and osteoporosis risk in White British men

Abstract

In this study, VDR gene *ApaI* (rs7975232), *BsmI* (rs 1544410) and *TaqI* (rs731236) genotypes were compared in men with osteoporosis and male controls. Osteoporosis affects around 20% of all men and overall mortality in the first year after hip fracture is significantly higher in men than women, yet the genetic basis of osteoporosis is less well studied in males. This study consisted of White British males; 69 osteoporosis patients and 122 controls. BMDs at the lumbar spine (vertebrae L1–L4) and hip (femur neck) were measured by dual-energy X-ray absorptiometry (DEXA). The VDR gene *ApaI*, *BsmI* and *TaqI* genotypes were determined by polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) and association analysis was carried out at genotype and haplotype level. Our study suggests that *TaqI* polymorphism CC genotype frequency is lower in controls and further analysis of genotypes and BMD revealed a significant effect of *TaqI* polymorphism on Lumbar spine BMD. Two haplotypes (GCC and AAT) were associated with increased osteoporosis risk. In conclusion, VDR gene *TaqI* polymorphism in recessive mode had a significant effect on lumbar spine BMD within our study. Haplotypes GCC and AAT increase the risk of osteoporosis among White British males.

Keywords: Bone mineral density (BMD), gender, osteoporosis, polymorphism, vitamin D receptor (VDR), haplotypes

Introduction

Osteoporosis (OP) is a common metabolic bone disease, characterised by a reduction in bone mineral density (BMD) and microarchitectural deterioration of bone tissue, consequently increasing bone fragility and fracture risk (Rachner et al. 2011). The prevalence of osteoporosis in UK males aged 50 years or more was estimated at 6.7% whereas the occurrence appeared approximately three times (22.1%) higher in females (Willson et al 2015). Yet, despite the lower prevalence of osteoporosis, men have higher morbidity and mortality rates after fracture (Kanis et al 2003).

Osteoporosis is a complex, multifactorial disease influenced by multiple risk factors including gender, age, ethnicity, lack of physical exercise, smoking, high alcohol consumption and low body mass (Rachner et al 2011). Family and twin studies (Spector et al 1995) have confirmed that genetics play a vital role in regulating BMD, with studies estimating that 50 to 85% of the variance in BMD is genetically determined (Ralston et al 2006). Genome wide association studies (GWAS) and candidate gene analyses have identified more than 100 loci associated with BMD, osteoporosis and osteoporotic fracture (OF) including the vitamin D receptor (VDR) (Grundberg et al. 2007; Zhang et al. 2018). Vitamin D and its cognate receptor (VDR) play a strong role in bone homeostasis and large studies have linked serum 25(OH)D levels, the main circulating metabolite of vitamin D, with BMD in both men and women (Uitterlinden et al 2002). The VDR gene is relatively large (at least 80 kb) with over 100 different polymorphisms. Four polymorphisms (*ApaI*, *BsmI*, *TaqI*, and *FokI*) of the VDR gene are frequently studied in association with BMD and osteoporosis and each loci is biallelic: *ApaI* (rs7975232 A/C); *BsmI* (rs1544410 A/G); *FokI* (rs228570 T/C) and *TaqI* (rs731236, T/C). Previously these allelic variants at the VDR loci have been designated by upper and lower case of the starting initial of the named loci e.g. *BsmI* (b and B),

TaqI (t and T), *ApaI* (a and A) and *FokI* (f and F).

With the higher numerical incidence of OP in females compared to males, most studies focus mainly on women, in particular older and/or post-menopausal women. As a result, the genetic associations between VDR and osteoporosis and BMD among males are largely unknown. A meta-analysis of *BsmI*, *TaqI*, *ApaI* and *FokI* VDR polymorphisms concluded that no clear association exists with OP in females (Zintzaras et al. 2006). Yet males presenting the 'baT' and 'BAt' haplotypes had a lower frequency of vertebral fractures than males with the 'bAT' haplotype (P<0.023) (Alvarez-Hernandez et al. 2003). Thus, the genetic effects on bone may be gender and site-specific, resulting in different genes regulating bone density at different skeletal sites. As genetic analyses show variable risk alleles and effects among different ethnicities, age groups and genders (Francis et al. 1997; Singh et al. 2013), smaller scale homogenous population studies are valuable to obtain a picture of inter-group differences (Singh et al. 2013).

An earlier study in a small subset of the current cohort showed no association between VDR polymorphism and bone density or fractional calcium (Francis et al. 1997). But a larger subset of the same cohort showed association between lumbar spine BMD and VDR *FokI* polymorphism (Kanan et al. 2000). This report focuses on a well characterised male sample from a single ethnicity.

Subjects and methods

Subjects

This study consisted of White British males; 69 patients with osteoporosis and 122 control subjects (Table 1). The recruitment details are published previously (Al-oanzi et al. 2008). Any control subjects with previous history of fractures were excluded. The patient group consisted of men with a BMD T-Score below -2.5 defined by the WHO (1994), either at the femoral neck or lumbar spine. All participants gave written informed consent and the study was approved by the Local Ethics Committees.

Genotyping

Three VDR polymorphisms (*ApaI*, *BsmI* and *TaqI*) were genotyped using published PCR-RFLP methods (Zintzaras et al. 2006; Singh et al. 2013). 10% of the samples were randomly repeated to ensure validity of the genotyping methods.

Statistical Methods

Means and standard deviations were calculated for age (years), height (cm), weight (kg), BMI (kg/m2) and BMD (g/cm). The data was tested for normality, but where data were not normally distributed Mann-Whitney U tests were carried out to assess differences between cases and controls. Allele frequencies were calculated by gene counting and departure from Hardy-Weinberg equilibrium (HWE) was tested for each population. The odds ratios and ANOVAs were used to assess associations between genotypes, anthropometric data and risk of osteoporosis. Linkage disequilibrium analysis and haplotype analysis was conducted using SNPSTATs and Chaplin software (Singh et al. 2013).

Results

The descriptive characteristics and minor allele frequencies (MAF) of VDR SNPs for both groups are presented in Table 1. The control subjects were significantly older than patients, but BMI was similar in both groups. As expected, the BMDs were significantly lower in cases compared to controls ($P<0.0001$ for both BMD sites). The MAF of three loci (rs1544410, rs7975232, rs7311236) were 0.46, 0.35 and 0.42 in controls and 0.52, 0.38, and 0.49 among patients. All loci were in Hardy-Weinberg equilibrium in both groups. Table 2 presents the genotypic, allelic and haplotypic associations and shows that minor (susceptible) alleles in each case increase the odds ratios in different combinations but the majority fail to achieve statistical significance. The CC genotype at *TaqI* polymorphism is significantly associated with osteoporosis (OR 2.13, CI 1.00-4.53, $P<0.05$) in the recessive model. These odds ratios are enhanced when adjusted for age and BMI. ANOVA analysis revealed a significant effect of rs731236 (TaqI) polymorphism on lumbar spine BMD. The CC genotype had significantly lower BMD (0.89+ 0.16) compared to CT (0.99+0.20) and TT (0.98+0.21) genotypes leading to overall significance ($P<0.05$).

Haplotypes results are presented in Table 2 along with their traditional nomenclature. Eight haplotypes were discernible but three (ACC, GAT and ACT) had frequencies lower than 5%, so were not considered for association analysis. AAC (BAt) haplotype was most common among controls and was used as the reference. GCC (bat) haplotype increased the risk of osteoporosis by 3.48 times in both crude and adjusted analyses (OR 3.48, CI 1.12-10.85, p<0.05). AAT (BAT) haplotype also increased the risk significantly in both analyses (Table 2). The associated haplotypes were analysed for the selection of a model (dominant, recessive, multiplicative) with best fit and parsimony according to AIC (Akaike information criterion) to determine which mode of

inheritance best describes its effect on BMD. The GCC susceptibility haplotype increases the risk of male osteoporosis with an effect of 2.29+0.72 (β+SEM) in multiplicative mode (*P*<0.001), while AAT haplotype increased the risk of osteoporosis with an effect of 1.84+0.67(β+SEM) in recessive mode (*P*<0.01).

Weak linkage disequilibrium (D') was observed between *BsmI -TaqI* (0.072) while *TaqI-ApaI* (0.414) and *BsmI-ApaI* (0.444) were of low/medium range.

Discussion

This small but homogenous study examined the role and relevance of VDR gene polymorphisms in a male osteoporosis population from the UK. Allele frequencies and genotype distribution of controls were comparable with the 1000 genome GBR population (IGSR 2018). Many studies of VDR gene are focused on osteoporosis in women and risk of fractures, but results are conflicting in respect of individual loci and haplotypes (Zintzaras et al. 2006; Zhang et al. 2018; Fang et al. 2005). In this study, risk alleles (MAF) at different loci increased the risk of osteoporosis, but it was only significant in recessive mode at *TaqI* polymorphism which has been observed in some studies among females, where CC (tt) was associated with osteoporosis (Zhang et al 2018). We observed a similar effect among males in this study showing a significant effect of *TaqI* polymorphism on LS-BMD. VDR *TaqI* polymorphism does affect the mRNA stability which can lead to changes in levels and biological functions of vitamin D.

The AAT (BAT) haplotype was significantly associated with the risk of male osteoporosis in this study. The other significant risk haplotype GCC (bat) is rare in most populations and its significant effect observed in this study requires further analysis at the genetic and structural level with a larger sample. Persisting considerable allelic

heterogeneity at VDR locus in relation to BMD instils ambiguity, leaving the picture regarding VDR haplotypes in relation to osteoporosis risk unclear. For instance, haplotypes GCT and GAT were associated with OP in Korean COPD patients (Kim et al 2015) but were absent in Swedish and Hong Kong osteoporotic males in te Global MrOS study (Grindberg et al 2007), which concluded that three haplotypes (GGT, ATC and GTT) were associated with vertebral fracture risk and lower FN BMD. Such incongruent relationships between VDR haplotypes and osteoporosis risk can have many reasons and require further large multiregional studies.

Recent GWAS focusing on BMD and fracture risk (Morris et al 2018, Kim 2019, Chesi et al 2019) have identified many novel loci for osteoporosis. Morris et al report sex heterogeneity within their analysis of UK Biobank samples with significant gender differences observed at 6 distinct loci at genome-wide significance. The six gender dependent loci affected differences in the magnitude and direction of association. Our analyses complement these findings that loci involved in osteoporosis may follow gender specific expression and further functional and clinical studies should be carried out on these differentially associated GWAS signals.

It has been shown that environmental parameters can influence an individual's measured BMD. Dietary supplementation with Vitamin D and/or Calcium across life can affect BMD and fracture risk (Tai et al 2015, Lloyd-Davies et al 2018). Therefore, in clinical association studies where environmental impact has not been fully recorded this may be a co-founding variable when assessing the output from single gene analyses and warrants further studies.

Overall this study highlights that there are specific VDR genotype and haplotype combinations which may modulate BMD in a gender specific manner and larger/comprehensive studies are required in male cohorts to disentangle the genetics of

VDR in osteoporosis.

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Table 1. General and genetic characteristics of the study population.

Variables	Osteoporotic Subjects	Normal Subjects	Total
Number	69	121	190
Age (Years)	58.96 ± 12.78	64.98 ± 10.06*	62.79 ± 11.49
BMI (Kg/m^2)	24.98 ± 4.40	25.68 ± 3.52	25.43 ± 3.88
Lumbar Spine BMD (g/cm^2)	0.78 ± 0.09	1.08 ± 0.16*	0.97 ± 0.20
Femoral neck BMD (g/cm^2)	0.69 ± 0.10	0.85 ± 0.11*	0.79 ± 0.13
rs731236 (MAF ± SE)[a]	0.49 ± 0.046	0.42 ± 0.030	0.45 ± 0.025
rs1544410 (MAF ± SE)[a]	0.52 ± 0.042	0.46 ± 0.030	0.48 ± 0.025
rs17879735 (MAF ± SE)[a]	0.38 ± 0.047	0.35 ± 0.029	0.37 ± 0.025

Values are mean ± SD, *P <0.001 vs. normal; two tailed, [a]MAF ± SE minor allele frequencies ± standard error.

Table 2 Genotype, allelic and haplotype numbers, frequencies and odds ratios for VDR loci.

Model	Genotype	Control No (%)	Patient No (%)	Crude OR (95% CI)	Crude P-value	Adjusted OR (95% CI) for Age and BMI	Adjusted *P* Value
				BsmI (rs1544410)			
	G/G	31 (26.3%)	11 (19%)	1.00 (ref)		1.00 (ref)	
Codominant	A/G	66 (55.9%)	34 (58.6%)	1.45 (0.65-3.24)	0.5	1.34 (0.56-3.21)	0.45
	A/A	21 (17.8%)	13 (22.4%)	1.74 (0.66-4.63)		2.01 (0.67-6.01)	
Dominant	G/G	31 (26.3%)	11 (19%)	1.00 (ref)	0.28	1.00 (ref)	0.36
	A/G-A/A	87 (73.7%)	47 (81%)	1.52 (0.70-3.30)		1.48 (0.64-3.43)	
Recessive	G/G-A/G	97 (82.2%)	45 (77.6%)	1.00 (ref)	0.47	1.00 (ref)	0.29
	A/A	21 (17.8%)	13 (22.4%)	1.33 (0.61-2.90)		1.63 (0.67-3.97)	
Log-additive	---	---	---	1.32 (0.82-2.14)	0.26	1.41 (0.82-2.45)	0.21
				ApaI (rs7975232)			
	A/A	48 (39.3%)	23 (40.4%)	1.00 (ref)		1.00 (ref)	
Codominant	A/C	62 (50.8%)	25 (43.9%)	0.84 (0.43-1.66)	0.47	0.81 (0.37-1.74)	0.21
	C/C	12 (9.8%)	9 (15.8%)	1.57 (0.58-4.24)		2.21 (0.71-6.87)	
Dominant	A/A	48 (39.3%)	23 (40.4%)	1.00 (ref)	0.9	1.00 (ref)	0.98
	A/C-C/C	74 (60.7%)	34 (59.6%)	0.96 (0.50-1.82)		0.99 (0.48-2.04)	
Recessive	A/A-A/C	110 (90.2%)	48 (84.2%)	1.00 (ref)	0.26	1.00 (ref)	0.09
	C/C	12 (9.8%)	9 (15.8%)	1.72 (0.68-4.35)		2.52 (0.87-7.15)	
Log-additive	---	---	---	1.12 (0.70-1.80)	0.64	1.25 (0.73-2.15)	0.41
				TaqI (rs731236)			
	T/T	35 (29.9%)	18 (28.1%)	1.00 (ref)		1.00 (ref)	
Codominant	C/T	65 (55.6%)	29 (45.3%)	0.87 (0.42-1.78)	0.14	0.96 (0.42-2.18)	0.11
	C/C	17 (14.5%)	17 (26.6%)	1.94 (0.81-4.69)		2.45 (0.84-6.85)	
Dominant	T/T	35 (29.9%)	18 (28.1%)	1.00 (ref)	0.8	1.00 (ref)	

	C/T-C/C	82 (70.1%)	46 (71.9%)	1.09 (0.56-2.14)		1.23 (0.56-2.67)	0.61
Recessive	T/T-C/T	100 (85.5%)	47 (73.4%)	1.00 (ref)	**0.050***	1.00 (ref)	0.04*
	C/C	17 (14.5%)	17 (26.6%)	**2.13 (1.00-4.53)**		**2.52 (1.06-6.02)**	
Log-additive	---	---	---	1.34 (0.86-2.10)	0.19	1.51 (0.90-2.55)	0.12

Haplotypes (order of SNPs, rs1544410 (*BsmI*) rs7975232 (*ApaI*) and rs731236 (*TaqI*))

Haplotype+

AAC (BAt)	26 (21.6%)	13 (18.0%)	Reference		Reference	
GAC (bAt)	20 (16.8%)	10 (14.8%)	0.86 (0.38-1.95)	0.87	0.80 (0.32-1.75)	0.77
GCC (bat)	5 (3.7%)	9 (13.3%)	**3.48 (1.12-10.85)**	**0.05***	**3.48 (1.12-10.85)**	**0.05***
AAT (BAT)	14 (11.8%)	19 (27.6%)	**2.90 (1.35-6.26)**	**0.009***	**2.88 (1.32-6.06)**	**0.008***
GCT (baT)	23 (19.3%)	13 (18.0%)	0.99 (0.46-2.10)	0.087	0.99 (0.46-2.10)	0.087

Other haplotypes had frequency less than 5%

*significant at 5% level, + Traditional name of haplotypes given in brackets