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

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

Melissa Kow[a], Elizabeth Akam[a], Puneetpal Singh[b], Monica Singh[b], Nick Cox[a], Jasvinder Singh Bhatti[c], Stephen P Tuck[d], Roger M Francis[e], Harish Datta[d] and Sarabjit Mastana[a*]

[a]Human Genomics Lab, School of Sport, Exercise and Heath Sciences, Loughborough University, Loughborough, Leicestershire, LE11 3TU, United Kingdom; [b]Department of Human Genetics, Punjabi University Patiala, Punjab India; [c]Department of Biotechnology, Sri Guru Gobind Singh College Chandigarh, India; [d]James Cook University Hospital, Middlesbrough, and Newcastle University, Newcastle upon Tyne, United Kingdom; [e]Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne, United Kingdom

* Corresponding author; Dr Sarabjit Mastana, SSEHS, Loughborough University, Loughborough, Leicestershire, LE11 3TU, United Kingdom: Phone +44 (0)1509 223041: Fax: +44 (0)1509 226301; E mail s.s.mastana@lboro.ac.uk

ORCID ID: Melissa Kow, Elizabeth Akam - 0000-0002-8784-3742; Puneetpal Singh - 0000-0002-2926-6764; Monica Singh, Nick Cox, Jasvinder Singh Bhatti, Stephen P Tuck, Roger M Francis, Harish Datta and Sarabjit Mastana - 0000-0002-9553-4886

Number of text pages (7 pages), plus bibliography (15 references, 2 pages) and number of tables (two)

Wordcount: Main text: 1461

Short title – Effects of VDR SNPs in male osteoporosis

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

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 increases 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 to 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 gender (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.

Materials and Methods

Materials

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 fails 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 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 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 male osteoporosis population from the UK. Allele frequencies and

genotype distribution of controls were comparable with1000 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 the similar effect among males in this study showing 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 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 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 requires 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 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 gender specific manner and larger/comprehensive studies are required in male cohorts to disentangle the genetics of VDR in osteoporosis.

References

Al-oanzi ZH, Tuck SP, Mastana SS, Summers GD, Cook DB, Francis RM, Datta HK. (2008) Vitamin D-binding protein gene microsatellite polymorphism influences BMD and risk of fractures in men. Osteoporos Int. 19:951-960

Alvarez-Hernández D, Naves M, Díaz-López JB, Gómez C, Santamaría I, Cannata-Andía JB, et al (2003) Influence of polymorphisms in VDR and COLIA1 genes on the risk of osteoporotic fractures in aged men. Kidney Int 63:S14–S18 . doi: 10.1046/j.1523-1755.63.s85.5.x

Chesi A, Wagley Y, Johnson ME, Manduchi E, Su C, Lu S, et al (2019) Genome-scale Capture C promoter interactions implicate effector genes at GWAS loci for bone mineral density. Nat Commun. Mar 19;10(1):1260. doi: 10.1038/s41467-019-09302-x.

Francis RM, Harrington F, Turner E, Papiha SS, Datta HK. (1997) Vitamin D receptor gene polymorphism in men and its effect on bone density and calcium absorption. Clin Endocrinol (Oxf). 46:83-86.

Grundberg E, Lau EM, Pastinen T, Kindmark A, Nilsson O, Ljunggren O, Mellström D, Orwoll E, Redlund-Johnell I, Holmberg A, et al (2007) Vitamin D receptor 3′ haplotypes are unequally expressed in primary human bone cells and associated with increased fracture risk: The MrOS study in Sweden and Hong Kong. J Bone Miner Res 22:832–840 . doi: 10.1359/jbmr.070317

Kanan RM, Varanasi SS, Francis RM, Parker L, Datta HK. (2000) Vitamin D receptor gene start codon polymorphism (FokI)and bone mineral density in healthy male subjects. Clin Endocrinol (Oxf). 53:93-98.

Kanis JA, Oden A, Johnell O, De Laet C, Jonsson B, Oglesby AK (2003) The components of excess mortality after hip fracture. Bone 32:468 473 . doi: 10.1016/S8756-3282(03)00061-91.

Kim SK. (2018) Identification of 613 new loci associated with heel bone mineral density and a polygenic risk score for bone mineral density, osteoporosis and fracture. PLoS One. Jul 26;13(7):e0200785. doi: 10.1371/journal.pone.0200785.

Kim SW, Lee JM, Ha JH, Kang HH, Rhee CK, Kim JW, Moon HS, Baek KH, Lee SH (2015) association between vitamin D receptor polymorphisms and osteoporosis in patients with COPD. Int J COPD 10:1809–1817 . doi: 10.2147/COPD.S91576

Lloyd-Davies LH, Jameson KA, Fuggle NR, Cooper C, Dennison EM, (2018) Vitamin D associated single nucleotide polymorphisms are associated with bone mass and microarchitecture in later life: findings from the Hertfordshire Cohort Study.

Rheumatology, Volume 57, Issue suppl_3, https://doi.org/10.1093/rheumatology/key075.561

Morris JA, Kemp JP, Youlten SE, Laurent L, Logan JG, Chai RC, et al (2019) An atlas of genetic influences on osteoporosis in humans and mice. Nat Genet. Feb;51(2):258-266. doi: 10.1038/s41588-018-0302-x.

Ralston SH, Crombrugghe B De, de Crombrugghe B (2006) Genetic regulation of bone mass and susceptibility to osteoporosis. Genes Dev 20:2492–2506 . doi: 10.1101/gad.1449506.contribution

Rachner TD, Khosla S, Hofbauer LC (2011) Osteoporosis: Now and the future. Lancet 377:1276–1287 . doi: 10.1016/S0140-6736(10)62349-5

Singh M, Singh P, Singh S, Juneja PK, Kaur T (2013) Vitamin D receptor (VDR) gene polymorphism influences the risk of osteoporosis in postmenopausal women of Northwest India. Arch Osteoporos 8:1–8 . doi: 10.1007/s11657-013-0147-y

Spector TD1, Keen RW, Arden NK, Morrison NA, Major PJ, Nguyen TV, Kelly PJ, Baker JR, Sambrook PN, Lanchbury JS, et al. (1995) Influence of vitamin D receptor genotype on bone mineral density in postmenopausal women: a twin study in Britain. BMJ 310:1357–60

Tai V, Leung W, Grey A, Reid IR, Bolland MJ. (2015) Calcium intake and bone mineral density: systematic review and meta-analysis. BMJ. 2015 Sep 29;351:h4183. doi: 10.1136/bmj.h4183

Uitterlinden AG, Fang Y, Bergink AP, van Meurs JB, van Leeuwen HP, Pols HA (2002) The role of vitamin D receptor gene polymorphisms in bone biology. Mol Cell Endocrinol 197:15–21

Willson T, Nelson SD, Newbold J, Nelson RE, LaFleur J (2015) The clinical epidemiology of male osteoporosis: A review of the recent literature. Clin Epidemiol 7:65–76 . doi: 10.2147/CLEP.S40966

Zhang L, Yin X, Wang J, Xu D, Wang Y, Yang J, Tao Y, Zhang S, Feng X, Yan C (2018) Associations between VDR Gene Polymorphisms and Osteoporosis Risk and Bone Mineral Density in Postmenopausal Women: A systematic review and Meta-Analysis. Sci Rep 8:1–16 . doi: 10.1038/s41598-017-18670-7

Zintzaras E, Rodopoulou P, Koukoulis GN (2006) ApaI, BsmI, FokI and TaqI polymorphisms in the vitamin D receptor (VDR) gene and the risk of Osteoporosis: A meta-analysis. Dis Markers 22:317–326 .

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)**			
Codominant	G/G	31 (26.3%)	11 (19%)	1.00 (ref)		1.00 (ref)	
	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)		1.00 (ref)	
	A/G-A/A	87 (73.7%)	47 (81%)	1.52 (0.70-3.30)	0.28	1.48 (0.64-3.43)	0.36
Recessive	G/G-A/G	97 (82.2%)	45 (77.6%)	1.00 (ref)		1.00 (ref)	
	A/A	21 (17.8%)	13 (22.4%)	1.33 (0.61-2.90)	0.47	1.63 (0.67-3.97)	0.29
Log-additive	---	---	---	1.32 (0.82-2.14)	0.26	1.41 (0.82-2.45)	0.21
				***ApaI* (rs7975232)**			
Codominant	A/A	48 (39.3%)	23 (40.4%)	1.00 (ref)		1.00 (ref)	
	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)		1.00 (ref)	
	A/C-C/C	74 (60.7%)	34 (59.6%)	0.96 (0.50-1.82)	0.9	0.99 (0.48-2.04)	0.98
Recessive	A/A-A/C	110 (90.2%)	48 (84.2%)	1.00 (ref)		1.00 (ref)	
	C/C	12 (9.8%)	9 (15.8%)	1.72 (0.68-4.35)	0.26	2.52 (0.87-7.15)	0.09
Log-additive	---	---	---	1.12 (0.70-1.80)	0.64	1.25 (0.73-2.15)	0.41
				***TaqI* (rs731236)**			
Codominant	T/T	35 (29.9%)	18 (28.1%)	1.00 (ref)	0.14	1.00 (ref)	

	C/T	65 (55.6%)	29 (45.3%)	0.87 (0.42-1.78)		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)	0.61
	C/T-C/C	82 (70.1%)	46 (71.9%)	1.09 (0.56-2.14)		1.23 (0.56-2.67)	
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