Disease Markers 22 (2006) 317–326
IOS Press

BsmI, TaqI, ApaI and FokI polymorphisms in the vitamin D receptor (VDR) gene and the risk of osteoporosis: A meta-analysis

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Abstract. A meta-analysis regarding BsmI, TaqI, ApaI and FokI polymorphisms in the vitamin D receptor (VDR) gene and their associations with osteoporosis in females is reported. The meta-analysis involved 14, seven, seven and three studies for BsmI, TaqI, ApaI and FokI polymorphisms, respectively. The studies were association studies with osteoporotic cases and controls free of osteoporosis that provided the genotype distribution of individual cases and controls. For the BsmI polymorphism, the allele contrast b vs. B showed heterogeneity among studies ($p < 0.01$, $I^2 > 50\%$) and the random effects (RE) pooled odds ratio (OR) was non-significant: 0.94 [95% confidence interval (CI) 0.63–1.38]. Caucasians, postmenopausal cases and studies with WHO diagnostic criteria showed no association under any genetic contrast. However, in East Asians, the OR for the dominant model [fixed effects OR = 0.14(95% CI 0.04–0.50) and RE OR = 0.16 (95% CI 0.03–0.84)] was significant, indicating prevention. Overall, for the TaqI, ApaI and FokI polymorphisms, the allele contrast showed heterogeneity and the pooled RE ORs were non-significant [OR = 1.06 (95% CI 0.71–1.60), OR = 0.99 (95% CI 0.72–1.37) and OR = 1.17 (95% CI 0.76–1.80), respectively]. The allele contrast for Caucasians, East Asians, postmenopausal cases and studies with WHO diagnostic criteria showed no association for TaqI, ApaI, and FokI. The allele contrast of homozygotes, and the recessive and dominant models the results followed the same pattern as the allele contrast. Therefore, the relationship between the VDR polymorphisms and osteoporosis remains an unresolved issue and other probable genetic-environmental risk factors interacting with the above polymorphisms should be investigated.

Keywords: VDR, osteoporosis, meta-analysis

1. Introduction

Osteoporosis is a systemic skeletal disease characterized by low bone mineral density (BMD) and microarchitectural deterioration of bone leading to increased bone fragility and high risk fracture. Twin and family studies have shown that BMD is influenced by genetic determinants up to 80%, which is the major predictor of osteoporosis [1–3].

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Among the multiple candidate genes so far investigated in relation to BMD and osteoporosis the vitamin D receptor (VDR) gene is the first [4] and the most controversial one [1,5]. Four polymorphisms (BsmI, TaqI, ApaI and FokI) of the VDR gene are the most frequently studied in association to BMD and osteoporosis. In the VDR gene cluster, the polymorphisms in each loci are detected with two alleles: BsmI (b and B), TaqI (t and T), ApaI (a and A) and FokI (f and F).

The genetic association studies that have investigated so far the association between osteoporosis, and the BsmI, TaqI, ApaI and FokI polymorphisms, provide some controversial or non-conclusive results, partly because each typically involved few cases and few con-

ISSN 0278-0240/06/$17.00

trols and, therefore, there was not enough information to demonstrate association. Furthermore, the interpretation is complicated by the fact that different populations, sampling strategies and number of loci included in the analyses have been used. In order to overcome the limitations of individual studies, and to resolve these controversial results, as well as to decrease the uncertainty of the effect size of estimated risk, a meta-analysis was conducted [6]. The meta-analysis estimated the effect of allele contrast, the contrast of homozygotes, and the contrasts for the dominant and recessive models. The consistency of genetic effects across populations from different ethnicities [7] and the effect of menopausal status were investigated. Furthermore, the heterogeneity between studies and the existence of potential bias were also investigated [8,9].

2. Methods

2.1. Selection of studies

All genetic association studies that investigated the association of the BsmI, TaqI, ApaI and FokI polymorphisms in the VDR gene with the development of osteoporosis published before December 2005 were considered in the meta-analysis. The studies were identified by extended computer based search of the PubMed database. The following searched criterion was used: ("VDR" or "BsmI" or "TaqI" or "ApaI" or "FokI" or "BsmI" or "Taq1" or "Apa1" or "Fok1") and ("BMD" or "Bone Mineral Density" or "Osteoporosis") was used. The retrieved publications were then read in their entirety in order to assess their appropriateness for inclusion in this meta-analysis. All references cited in the publications were also reviewed to identify additional published work not identified by PubMed database search. Abstracts, case reports, editorials and review articles were excluded. The search was restricted to articles in English, French, and Spanish.

Genetic association studies that determine the distribution of the BsmI, TaqI, ApaI and FokI genotypes in cases with osteoporosis, and in a control group, were eligible for inclusion in the meta-analysis. Cases were considered as osteoporotic female patients after diagnosis using valid published criteria (see Table 1). The control group consisted of healthy or non-osteoporotic diseased subjects. Only studies that have used validated genotyping methods were considered. The distribution of the genotypes in the control group was tested for Hardy-Weinberg equilibrium (HWE; $p \geqslant 0.05$) [10]. Studies based on pedigree data that investigate linkage [8,11] were excluded.

2.2. Data extraction

From each study the following information was extracted: first author, journal, year of publication, ethnicity of study population, demographics, menopausal status, method of BMD measurement, site of BMD measurement, matching, validity of the genotyping method, blindness of genotyping, and the number of cases and controls for each BsmI, TaqI, ApaI or FokI genotype. The frequencies of the alleles were calculated from the corresponding genotype distributions.

2.3. Meta-analysis

Prior to the main analysis, the significance of the associations for: i) the allele contrast, ii) the contrast of homozygotes, iii) the recessive and iv) dominant models. All associations were indicated as odds ratios (ORs) with the corresponding 95% confidence interval (CI). Based on the individual ORs, a pooled OR was estimated.

The heterogeneity between studies was tested using the Q-statistic [8,12]. When $p < 0.10$ then the heterogeneity was considered statistically significant. Heterogeneity was quantified with the I^2 metric, which is independent of the number of studies in the meta-analysis. I^2 takes values between 0% and 100% with higher values denoting greater degree of heterogeneity [13]. The pooled OR was estimated using fixed effects (FE; Mantel-Haenszel) and random effects (RE; DerSimonian and Laird) models [14]. The random effects model assumes a genuine diversity in the results of various studies and incorporates in the pooled OR the between studies variance. Therefore, when there is heterogeneity between studies the pooled OR was estimated using the RE model. Adjusted estimates of OR were considered whenever possible in a separate analysis. A cumulative meta-analysis and recursive cumulative meta-analysis were carried out in order to evaluate the trend of pooled OR for the allele contrast in time [15,16]. A differential magnitude of effect in large versus small studies (bias) for the allele contrast was checked using the Egger regression test for funnel plot asymmetry and the Begg-Mazumdar test [17,18].

The meta-analysis consisted of the main (overall) analysis which includes all available data, the subgroup analyses by ethnicity, menopausal status and diagnostic criteria, and sensitivity analysis which examines the effect of excluding specific studies (studies with controls not in HWE [10]).

Analyses were performed using Meta-Analyst (Joseph Lau, Boston, Massachusetts, USA 1998), and CVF90 with IMSL library as employed in previous studies [19–21]. The distribution of the genotypes in the control group was tested whether it is in Hardy-Weinberg equilibrium using an exact test [10] implemented by GDA software [22].

3. Results

3.1. Eligible studies

The literature review identified 335 titles that met the search criteria. Data from 14 studies that investigated the association between any of the BsmI, TaqI, ApaI and FokI polymorphisms and osteoporosis met the inclusion criteria and were included in the meta-analysis.

Fourteen studies dealt with BsmI [2,23–35], six with TaqI [2,24,28,30–32,35], six with ApaI [2,24,28,30–32,35] and three with FokI [30,31,33]. The studies were published between 1994 and 2004. In all studies, the cases were well defined following valid criteria, and the controls were healthy or non-osteoporotics diseased subjects. For the determination of the genetic polymorphisms of BsmI, TaqI, ApaI and FokI validated genotyping methods were used in all studies; namely, PCR and restriction of the PCR product with the enzyme corresponding to each polymorphism. In eight studies, the controls were age or sex matched [2,25–27,30–32,35], and in five studies, they were matched in years since menopause [2,26,31,32,35]. Studies were conducted in various populations of racial descent: nine involved Caucasians [23,24,26,28–32,35], two East Asians [25, 27] and three other ethnicities (Mexican American, Latino, Turkish) [2,33,34]. Eleven studies reported that the cases were postmenopausal [2,24,26–28,30–35]. Nine studies reported diagnosis of cases based on WHO criteria [2,25,27,30–33,35]. A list of all the details abstracted from these studies is provided in Table 1.

3.2. Summary statistics

Overall, the studies provided 898/1594 cases/controls for BsmI, 500/776 cases/controls for TaqI, 540/1188 cases/controls for ApaI, and 153/246 cases/controls for FokI. In all polymorphisms there were excess of heterozygotes. In cases and controls, the alleles b, T, A, and F were the most common for BsmI, TaqI, ApaI, and FokI polymorphism, respectively (Table 1).

In seven studies [2,27,29,32–35] for the BsmI, in one study [28] for the TaqI, in four studies [2,28,32, 35] for the ApaI, and in one study [33] for the FokI, the distribution of the genotypes in control group was not in Hardy-Weinberg equilibrium ($p < 0.05$), indicating genotyping errors and/or population stratification [36]. Therefore, a sensitivity analysis was carried out for these studies.

One study [28] reported linkage disequilibrium for BsmI, ApaI, TaqI polymorphisms, and one study [30] for FokI, BsmI, ApaI, TaqI polymorphisms.

3.3. Main results, subgroup and sensitivity analyses

Table 3 and Fig. 1 show the results for the association between the different polymorphisms and the risk of osteoporosis, and the homogeneity significance.

For the BsmI polymorphism and its relationship to osteoporosis the allele contrast b vs. B showed heterogeneity among studies ($p < 0.01$, $I^2 > 50\%$) and the pooled OR was non-significant: RE OR = 0.94 (95% CI 0.63–1.38). In subgroup analysis, the RE pooled ORs for the Caucasians and East Asians were not significant [OR = 1.11 (95% CI 0.71–1.73) and OR = 0.35 (95% CI 0.06–2.01)], respectively. In addition, the studies reported cases as postmenopausal and the studies with WHO diagnostic criteria produced non-significant association [RE OR = 0.86 (95% CI 0.57–1.30) and RE OR = 0.92 (95% CI 0.54–1.56)]. Overall, in Caucasians, in postmenopausal cases and in studies with WHO diagnostic criteria the contrast of homozygotes (bb vs. BB), the recessive and dominant models for allele b produced non-significant results. In East Asians, the ORs for the contrast of homozygotes [FE OR = 0.12 (95% CI 0.03–0.42) and RE OR = 0.15 (95% CI 0.02–1.06)] and the dominant model [FE OR = 0.14 (95% CI 0.04–0.50) and RE OR = 0.16 (95% CI 0.03–0.84)] were significant, indicating prevention.

Overall, for the TaqI, ApaI and FokI polymorphisms and its relationship to osteoporosis the allele contrast showed heterogeneity among studies and the RE pooled ORs were non-significant [OR = 1.06 (95% CI 0.71–1.60), OR = 0.99 (95% CI 0.72–1.37) and OR = 1.17 (95% CI 0.76–1.80), respectively]. The subgroup analysis for Caucasians, East Asians, postmenopausal cases and studies with WHO diagnostic criteria produced non-significant results. The contrast of homozygotes, and the recessive and dominant models the results followed the same pattern as the allele contrast for TaqI, ApaI, and FokI. The sensitivity analysis did not change the results for ever contrast and, hence, no significant association was detected.

Table 1
Characteristics of eligible studies considered in the meta-analysis

			Cases						Controls				
Author, Year	**Racial descent**	**Polymor-phisms**	**N**	**Age** (Mean±SD)yrs (min-max)yrs	**Meno-pause**	**BMD site**	**Diagnosis M**	**atching**	**N**	**Healthy**	**Age** (Mean±SD)yrs (min-max)yrs	**Meno-pause**	**BMD site**
Melhus, 1994	Caucasian	BsmI	70	Mean=70.8yrs	Ne	LS-hip	LS BMD <0,91 gr/cm2 defined as fracture threshold	Ne	76	Ne	Mean=69.8yrs	Ne	LS-hip
Riggs, 1995	Caucasian	BsmI, ApaI, TaqI	43	(53-76)yrs	PSM	LS-hip	LS BMD<0,85 gr/cm2 and fractures	Ne	127	Yes	Ne	Pre-PSM	Ne
Lim, 1995	East Asian	BsmI	72	(55-72)yrs	Ne	LS-hip	WHO	Ne	70	Yes	(35.0±2.3)yrs	Ne	Ne
Yanagi, 1996	East Asian	BsmI	46	(65.0±8.8)yrs	PSM	LS	WHO	Age	66	Yes	(64.9±6.3)yrs	PSM	LS
Houston, 1996	Caucasian	BsmI	44	(66.0±0.85)yrs	PSM	LS-hip	WHO	Age, gender, time from menopause	44	Yes	(65.3±0.9)	PSM	LS-hip
Vandevyver, 1997	Caucasian	BsmI, ApaI, TaqI	84	(66.6±8.4)yrs	PSM	LS-hip	femoral neck density Z score non-normally distributed	Ne 807		Yes	(75.5±5.0)yrs	PSM	LS-hip
Gennari, 1998	Caucasian	BsmI, ApaI, TaqI	176	(58.2±0.6)yrs	PSM	LS	WHO	Age, weight, years since menopause	144	Yes	(57.1±0.7)yrs	PSM	LS
Poggi, 1999	Caucasian	BsmI	40	Ne	Ne	LS	LS BMD<0,750 gr/cm2	Ne	10	Ne	Ne	Ne	LS
Zajickova , 2002	Caucasian	BsmI, ApaI, TaqI, FokI	65	(63.6±7.8)yrs	PSM	LS-hip	WHO	BMI, years since menopause	33	Yes	(60.1±10.3)yrs	PSM	LS-hip
Langdahl, 2000	Caucasian	BsmI, ApaI, TaqI, FokI	110	(58.2±6.4)yrs	PSM	LS-hip	WHO	Age	153	Yes	(56.2±7.7)yrs	PSM	LS-hip
Douroudis, 2003	Caucasian	BsmI, ApaI, TaqI	35	(61.37±0.96)yrs	PSM	Distal forearm	WHO	Age, BMI, years after menopause	44	Yes	(58.7±1.0)yrs	PSM	Distal forearm
Lisker, 2003	Mexican	BsmI, FokI	65	(65.2±6.8)yrs	PSM	LS-hip	WHO	Ne	57	Yes (10% OP)	(56.5±6.0)yrs	PSM	LS-hip
Borjas-Fajardo, 2003	Latino	BsmI	54	(61.7±8.3)yrs	PSM	Ne	Positive densitometry	Ne	55	Yes	(53.6±6.4)yrs	PSM	Ne
Duman, 2004	Turkish	BsmI, ApaI, TaqI	57	(53.16±1.31)yrs	PSM	LS-hip	WHO	Age, years of menopause, BMI	66	Yes	(52.6±1.7)yrs	PSM	LS-hip

Ne=non-extractable, SD=standard deviation, LS=lumbar spine, BMD=Bone Mineral Density, WHO=World Health Organization, BMI=body mass index, PSM=post-menopausal, Pre=pre-menopausal, OP=osteopenetics

Table 2
The genotype distribution and the allelic frequency of the (a) BsmI, (b) TaqI, (c) ApaI and (d) FokI VDR polymorphisms for all studies with osteoporotic cases and non-osteoporotic controls

(a)

Studies	Distribution of BsmI VDR genotype						Frequency of BsmI VDR alleles			
First author, year	bb		bB		BB		b		B	
	Cases N(%)	Controls N(%)	Cases N(%)	Controls N(%)	Cases N(%)	Controls N(%)	Cases N(%)	Controls N(%)	Cases N(%)	Controls N(%)
Melhus, 1994	27(38)	7(9.)	29(41)	35(46)	14(20)	34(44)	83(59)	49(32)	57(40)	103(67)
Riggs, 1995	11(27)	48(37)	20(50)	61(47)	9(22)	20(15)	42(52)	157(60)	38(47)	101(39)
Lim, 1995	61(84)	60(85)	9(12)	9(12)	2(2)	1(1)	131(90)	129(92)	13(9)	11(7)
Houston, 1996	17(38)	16(36)	19(43)	19(43)	8(18)	9(20)	53(60)	51(57)	35(39)	37(42)
Yanagi, 1996	22(47)	57(86)	12(26)	7(10)	12(26)	2(3)	56(60)	121(91)	36(39)	11(8)
Vandevyver, 1997	24(27)	203(29)	50(58)	368(52)	12(13)	127(18)	98(56)	774(55)	74(43)	622(44)
Gennari, 1998	23(14)	49(36)	92(59)	76(55)	40(25)	11(8.)	138(44)	174(63)	172(55)	98(36)
Poggi, 1999	1(10)	8(20)	7(70)	28(70)	2(20)	4(10)	9(45)	44(55)	11(55)	36(45)
Langdahl, 2000	19(23)	21(26)	38(47)	34(42)	23(28)	25(31)	76(47)	76(47)	84(52)	84(52)
Zajickova, 2002	20(30)	10(30)	24(36)	13(39)	21(32)	10(30)	64(49)	33(50)	66(50)	33(50)
Douroudis, 2003	20(57)	5(11)	12(34)	29(65)	3(8.)	10(22)	52(74)	39(44)	18(25)	49(55)
Lisker, 2003	34(51)	6(10)	17(25)	38(66)	15(22)	13(22)	85(64)	50(43)	47(35)	64(56)
Fajardo, 2003	6(11)	8(14)	20(37)	36(65)	28(51)	11(20)	32(29)	52(47)	76(70)	58(52)
Duman, 2004	3(4)	7(10)	54(72)	42(63)	18(24)	17(25)	60(40)	56(42)	90(60)	76(57)
Total	288(32)	505(31)	403(44)	795(49)	207(23)	294(18)	979(54)	1805(56)	817(45)	1383(43)

(b)

Studies	Distribution of TaqI VDR genotype						Frequency of TaqI VDR alleles			
First author, year	tt		tT		TT		t		T	
	Cases N(%)	Controls N(%)	Cases N(%)	Controls N(%)	Cases N(%)	Controls N(%)	Cases N(%)	Controls N(%)	Cases N(%)	Controls N(%)
Riggs, 1995	7(17)	20(15)	23(56)	57(43)	11(26)	53(40)	37(45)	97(37)	45(54)	163(62)
Vandevyver, 1997	5(10)	34(11)	30(65)	159(55)	11(23)	91(32)	40(43)	227(39)	52(56)	341(60)
Gennari, 1998	40(25)	11(7)	87(54)	71(49)	33(20)	62(43)	167(52)	93(32)	153(47)	195(67)
Langdahl, 2000	14(17)	13(17)	41(52)	34(45)	23(29)	28(37)	69(44)	60(40)	87(55)	90(60)
Zajickova, 2002	11(16)	8(24)	31(47)	14(42)	23(35)	11(33)	53(40)	30(45)	77(59)	36(54)
Douroudis, 2003	3(8)	9(20)	13(37)	27(61)	19(54)	8(18)	19(27)	45(51)	51(72)	43(48)
Duman, 2004	10(13)	15(22)	42(56)	28(42)	23(30)	23(34)	62(41)	58(43)	88(58)	74(56)
Total	90(18)	110(14)	267(53)	390(50)	143(28)	276(35)	447(44)	610(39)	553(55)	942(60)

(c)

Studies	Distribution of ApaI VDR genotype						Frequency of ApaI VDR alleles			
First author, year	aa		aA		AA		a		A	
	Cases N(%)	Controls N(%)	Cases N(%)	Controls N(%)	Cases N(%)	Controls N(%)	Cases N(%)	Controls N(%)	Cases N(%)	Controls N(%)
Riggs, 1995	9(22)	31(24)	19(47)	59(46)	12(30)	38(29)	37(46)	121(47)	43(53)	135(52)
Vandevyver, 1997	22(25)	127(18)	45(51)	375(53)	20(22)	197(28)	89(51)	629(44)	85(48)	769(55)
Gennari, 1998	11(6)	26(18)	81(50)	84(58)	68(42)	34(23)	103(32)	136(47)	217(67)	152(52)
Langdahl, 2000	12(15)	17(22)	44(56)	32(43)	22(28)	25(33)	68(43)	66(44)	88(56)	82(55)
Zajickova, 2002	9(13)	6(18)	33(50)	17(51)	23(35)	10(30)	51(39)	29(43)	79(60)	37(56)
Douroudis, 2003	10(28)	1(2)	14(40)	26(59)	11(31)	17(38)	34(48)	28(31)	36(51)	60(68)
Duman, 2004	6(8)	6(9)	56(74)	45(68)	13(17)	15(22)	68(45)	57(43)	82(54)	75(56)
Total	79(14)	214(18)	292(54)	638(53)	169(31)	336(28)	450(41)	1066(44)	630(58)	1310(55)

(d)

Studies	Distribution of FokI VDR genotype						Frequency of FokI VDR alleles			
First author, year	Ff		f F		FF		f		F	
	Cases N(%)	Controls N(%)	Cases N(%)	Controls N(%)	Cases N(%)	Controls N(%)	Cases N(%)	Controls N(%)	Cases N(%)	Controls N(%)
Langdahl, 2000	9(22)	31(24)	19(47)	59(46)	12(30)	38(29)	37(46)	121(47)	43(53)	135(52)
Zajickova, 2002	12(15)	17(22)	44(56)	32(43)	22(28)	25(33)	68(43)	66(44)	88(56)	82(55)
Lisker, 2003	10(28)	1(2)	14(40)	26(59)	11(31)	17(38)	34(48)	28(31)	36(51)	60(68)
Total	31(20)	49(19)	77(50)	117(47)	45(29)	80(32)	139(45)	215(43)	167(54)	277(56)

Table 3
Odds ratios (OR) with the corresponding 95% confidence interval (CI) and heterogeneity results (I^2 and p-values of Q-test) for the genetic contrasts of (a) BsmI, (b) TaqI, (c) ApaI, and (d) FokI VDR polymorphisms for osteoporosis

(a)

Contrast for BsmI	Population	Fixed effects OR(95%CI)	Random effects OR(95%CI)	I^2 (%)	p-value Q-test
b vs. B	All	0.93(0.82-1.06)	0.94(0.63-1.38)	87	<0.01
	All in HWE	1.15(0.96-1.38)	1.13(0.79-1.62)	71	<0.01
	All WHO criteria	0.85(0.72-1.00)	0.92(0.54-1.56)	87	<0.01
	Caucasians	1.00(0.86-1.16)	1.11(0.71-1.73)	86	<0.01
	East Asians	0.31(0.18-0.53)	0.35(0.06-2.01)	90	<0.01
	Postmenopausal	0.84(0.73-0.97)	0.86(0.57-1.30)	87	<0.01
	Cauc. Postmen. WHO	0.84(0.68-1.03)	1.08(0.57-2.05)	88	<0.01
bb vs. BB	All	0.88(0.67-1.14)	0.86(0.40-1.85)	83	<0.01
	All in HWE	1.36(0.94-1.95)	1.28(0.64-2.59)	68	<0.01
	All WHO criteria	0.69(0.49-0.97)	0.80(0.29-2.22)	84	<0.01
	Caucasians	0.99(0.73-1.35)	1.14(0.46-2.87)	85	<0.01
	East Asians	0.12(0.03-0.42)	0.15(0.02-1.06)	50	0.16
	Postmenopausal	0.71(0.54-0.95)	0.75(0.35-1.62)	82	<0.01
	Cauc. Postmen. WHO	0.69(0.45-1.03)	1.03(0.28-3.75)	87	<0.01
Recessive model	All	0.98(0.80-1.20)	1.05(0.57-1.93)	85	<0.01
	All in HWE	1.13(0.86-1.48)	1.15(0.71-1.88)	64	0.01
	All WHO criteria	0.88(0.68-1.14)	1.02(0.43-2.42)	89	<0.01
	Caucasians	0.99(0.78-1.25)	1.20(0.61-2.35)	84	<0.01
	East Asians	0.36(0.19-0.67)	0.37(0.06-2.25)	87	<0.01
	Postmenopausal	0.86(0.69-1.07)	0.94(0.49-1.82)	86	<0.01
	Cauc. Postmen. WHO	0.83(0.60-1.15)	1.17(0.43-3.19)	87	<0.01
Dominant model	All	0.83(0.67-1.04)	0.79(0.49-1.29)	74	<0.01
	All in HWE	1.30(0.95-1.78)	1.24(0.81-1.89)	39	0.13
	All WHO criteria	0.72(0.54-0.96)	0.76(0.43-1.35)	67	<0.01
	Caucasians	1.01(0.77-1.31)	1.03(0.58-1.81)	73	<0.01
	East Asians	0.14(0.04-0.50)	0.16(0.03-0.84)	29	0.23
	Postmenopausal	0.72(0.57-0.91)	0.72(0.44-1.18)	71	<0.01
	Cauc. Postmen. WHO	0.74(0.51-1.06)	0.90(0.41-1.97)	74	<0.01

(b)

Contrast for TaqI	Population	Fixed effects OR(95%CI)	Random effects OR(95%CI)	I^2 (%)	p-value Q-test
t vs. T	All	1.24(1.04-1.48)	1.06(0.71-1.60)	80	<0.01
	All in HWE	1.26(1.04-1.52)	1.04(0.64-1.69)	83	<0.01
	All WHO criteria	1.24(1.01-1.51)	0.97(0.54-1.76)	87	<0.01
	Caucasians	1.30(1.08-1.57)	1.09(0.69-1.73)	82	<0.01
	Caucasians WHO	1.33(1.06-1.66)	0.98(0.47-2.07)	87	<0.01
tt vs. TT	All	1.47(1.03-2.11)	1.11(0.47-2.62)	79	<0.01
	All in HWE	1.50(1.03-2.20)	1.08(0.40-2.95)	82	<0.01
	All WHO criteria	1.48(0.98-2.23)	0.97(0.29-3.29)	86	<0.01
	Caucasians	1.67(1.13-2.48)	1.21(0.45-3.20)	80	<0.01
	Caucasians WHO	1.76(1.11-2.70)	1.05(0.23-4.78)	89	<0.01
Recessive model	All	1.19(0.86-1.65)	0.98(0.52-1.85)	69	<0.01
	All in HWE	1.23(0.87-1.75)	0.98(0.47-2.06)	74	<0.01
	All WHO criteria	1.25(0.86-1.82)	0.94(0.38-2.32)	79	<0.01
	Caucasians	1.37(0.96-1.95)	1.09(0.55-2.17)	68	<0.01
	Caucasians WHO	1.53(1.01-2.33)	1.09(0.38-3.12)	80	<0.01
Dominant model	All	1.45(1.12-1.88)	1.22(0.70-2.13)	76	<0.01
	All in HWE	1.44(1.09-1.90)	1.16(0.60-2.25)	80	<0.01
	All WHO criteria	1.38(1.02-1.86)	1.04(0.49-2.31)	84	<0.01
	Caucasians	1.49(1.13-1.97)	1.21(0.62-2.33)	79	<0.01
	Caucasians WHO	1.42(1.02-1.97)	0.98(0.35-2.77)	88	<0.01

Table 3, continued

(c)

Contrast for ApaI	Population	Fixed effects OR(95%CI)	Random effects OR(95%CI)	I^2 (%)	p-value Q-test
a vs. A	All	0.94(0.80-1.11)	0.99(0.72-1.37)	72	<0.01
	All in HWE	0.93(0.69-1.24)	0.93(0.69-1.24)	0	0.91
	All WHO criteria	0.83(0.68-1.02)	0.95(0.62-1.46)	75	<0.01
	Caucasians	0.93(0.78-1.10)	0.98(0.68-1.42)	76	<0.01
	Caucasians WHO	0.78(0.62-0.97)	0.92(0.54-1.58)	79	<0.01
aa vs. AA	All	0.91(0.64-1.29)	0.96(0.45-2.07)	74	<0.01
	All in HWE	0.81(0.44-1.47)	0.81(0.44-1.47)	0	0.92
	All WHO criteria	0.67(0.42-1.05)	0.88(0.30-2.59)	75	<0.01
	Caucasians	0.90(0.63-1.28)	0.95(0.40-2.27)	78	<0.01
	Caucasians WHO	0.62(0.38-1.01)	0.86(0.23-3.21)	80	<0.01
Recessive model	All	0.94(0.69-1.27)	0.92(0.50-1.71)	69	<0.01
	All in HWE	0.74(0.44-1.24)	0.74(0.44-1.24)	0	0.80
	All WHO criteria	0.72(0.48-1.08)	0.84(0.35-2.00)	69	0.01
	Caucasians	0.95(0.69-1.30)	0.94(0.47-1.90)	74	<0.01
	Caucasians. WHO	0.70(0.45-1.09)	0.88(0.30-2.61)	76	<0.01
Dominant model	All	0.91(0.71-1.16)	0.98(0.65-1.47)	58	0.03
	All in HWE	1.05(0.67-1.64)	1.05(0.67-1.64)	0	0.68
	All WHO criteria	0.78(0.57-1.06)	0.92(0.52-1.61)	65	0.02
	Caucasians	0.87(0.67-1.13)	0.93(0.59-1.46)	62	0.02
	Caucasians WHO	0.71(0.51-0.99)	0.83(0.44-1.58)	68	0.03

(d)

Contrast For FokI	Population	Fixed effects OR(95%CI)	Random effects OR(95%CI)	I^2 (%)	p-value Q-test
f vs. F	All	1.13(0.84-1.52)	1.17(0.76-1.80)	50	0.14
	All in HWE	0.96(0.69-1.34)	0.96(0.69-1.34)	0	0.99
	All WHO criteria	1.13(0.84-1.52)	1.17(0.76-1.81)	50	0.14
	Caucasians	0.96(0.69-1.34)	0.96(0.69-1.34)	0	0.99
ff vs. FF	All	1.30(0.71-2.36)	1.55(0.43-5.59)	69	0.04
	All in HWE	0.86(0.43-1.69)	0.86(0.43-1.69)	0	0.84
	All WHO criteria	1.30(0.71-2.36)	1.55(0.43-5.59)	69	0.04
	Caucasians	0.86(0.43-1.69)	0.86(0.43-1.69)	0	0.84
Recessive model	All	1.13(0.67-1.89)	1.46(0.38-5.57)	77	0.01
	All in HWE	0.74(0.41-1.34)	0.74(0.41-1.33)	0	0.51
	All WHO criteria	1.13(0.67-1.89)	1.46(0.38-5.57)	77	0.01
	Caucasians	0.74(0.41-1.34)	0.74(0.41-1.33)	0	0.51
Dominant model	All	1.20(0.76-1.88)	1.20(0.76-1.88)	0	0.83
	All in HWE	1.15(0.69-1.93)	1.15(0.69-1.92)	0	0.60
	All WHO criteria	1.20(0.76-1.88)	1.20(0.76-1.88)	0	0.83
	Caucasians	1.15(0.69-1.93)	1.15(0.69-1.92)	0	0.60

3.4. Potential bias

None of the studies included in the meta-analysis stated that genotyping was performed blinded to clinical status. Overall, for the BsmI polymorphism, the cumulative meta-analysis and recursive cumulative meta-analysis for the allelic contrast showed that RE OR declined from 3.06 in 1994 (first study) to 0.80 in 1996 (relative change $= -74\%$) and then increased to 0.94 in 2004 (relative change $= +18\%$). For the TaqI polymorphism, the RE OR declined from 1.38 in 1995 to 1.25 in 1997 (relative change $= -9\%$) and then increased to 1.58 in 1998 (relative change $= +26\%$); a downward trend in the period 1998–2004 existed (OR $= 1.06$ in 2004; relative change $= -33\%$). For the ApaI polymorphism, the RE OR was non-significant in the studied period, however, the magnitude of RE OR increased from 0.96 in 1995 to 1.18 in 1997 (relative change $= 23\%$) and then declined to 0.99 in 2004 (relative change $= -16\%$). The Egger test and the Begg-Mazumdar test indicated that there is no differential magnitude of effect in large versus small studies for the BsmI polymorphism ($p = 0.90$ and $p = 0.52$, respectively).

4. Discussion

The aetiology of developing osteoporosis is still unknown, however, several researchers have shown

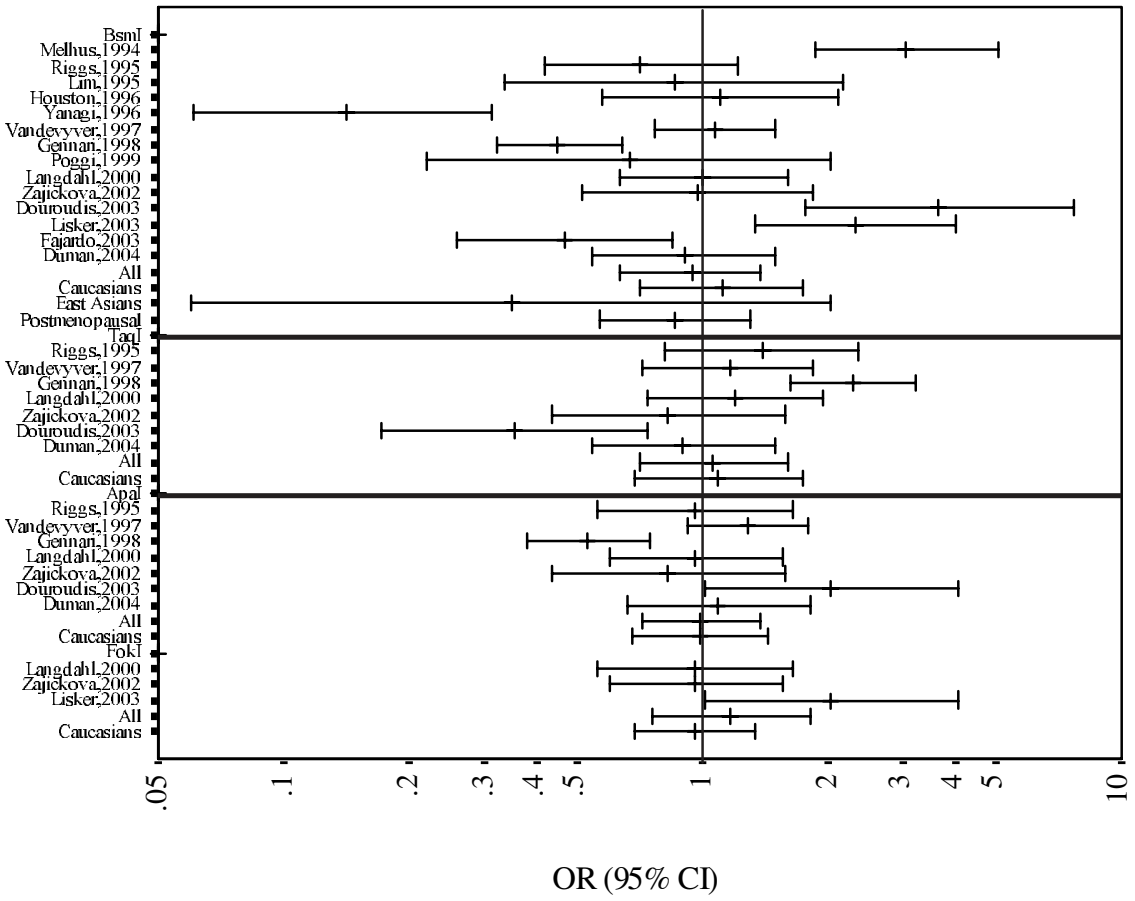

Fig. 1. The allele contrast for BsmI (b vs B), TaqI (t vs T), ApaI (a vs A), and FokI (f vs F) polymorphisms in the VDR gene. Each study is shown by an odds ratio (OR) estimate with the corresponding 95% confidence interval (CI). The random effects pooled odds ratios are shown. The horizontal axis is plotted on a log scale.

the importance of age, gene-environment interactions, gene-gene interactions and life-style in the development of osteoporosis [1,37]. Most research carried out so far deals with the VDR gene and the fact that single point mutations in the gene are known to alter metabolic activity [1]. In order to partly cover the main limitation of genetic association studies, namely, low sample sizes in single studies, a meta-analysis offers a robust tool. The strength of the present analysis, however, is based on the aggregation of published case-control studies, thus there is more information for investigating the effect of the allele under investigation than the individual studies [38]. Although this meta-analysis involved a considerable amount of subjects, the investigation of the genetic associations should be based on large population studies with similar study designs. The results of this meta-analysis depended on the study design and the inclusion criteria of the cases and the controls in each study. The cases and controls involved in the meta-analysis were well defined with similar inclusion criteria, although they unavoidably cover a spectrum of disease in terms of clinical, demographic and life-style or dietary data [37]. Our meta-analysis was based on unadjusted estimates, although, a more precise analysis could be performed if adjusted (e.g. by age, dietary intake, BMI) estimates were provided in the studies.

In all polymorphisms, there is excess of homozygotes. The main and subgroup analyses in Caucasians and postmenopausal cases for the allele contrast, the recessive and dominant models for all polymorphisms produced non-significant results, and heterogeneity ranged from none to high. The genetic effects across the different ethnicities were not consistent: In East Asians, it seems that BsmI is a preventive factor of osteoporosis under a recessive model for allele b, however, this result was based on only two studies, and any inferences should be with cautious. The meta-analysis included papers in English, Spanish and French. How-

ever, it is known, that the most clear-cut data have been coming from Asian countries, such as Japan and Korea. Thus, the analysis may have missed some papers in Japanese or Korean dealing with the association of VDR gene polymorphisms and osteoporosis. There is a consistence in genetic effects across the diagnostic criteria (overall studies and Caucasian postmenopausal cases with WHO diagnostic criteria) since the effects were non-significant and they did not deviate substantially from the main analysis. The meta-analysis indicated no potential bias: there was no differential magnitude of effect in large versus small studies.

A published meta-analysis [39] investigated the association between BsmI and BMD based on mean differences in BMD level for each genotype, and involved studies published till 2000, whereas, the present meta-analysis investigated the risk of osteoporosis based on genotype distribution of cases and controls from studies published till December 2005. Cohorts that provided an average of BMD for each genotype were not considered in the meta-analysis since risk of osteoporosis cannot be calculated [40].

The main benefit for conducting this meta-analysis was to decrease the uncertainty of the effect size of estimated risk, and to provide evidence (positive or negative): For example, the allele contrast b vs. B indicated that the change in odds would be less than 49% or more than 47% conferring risk or protection from osteoporosis. The accumulated evidence has excluded the presence of an association between the VDR polymorphisms and the risk of osteoporosis, but an association may exist in East Asians, in particular for BsmI polymorphism. The lack of association between osteoporosis and candidate genes such as VDR, and the discrepancy of results might be due to other loci that are probably in linkage disequilibrium and affect the susceptibility to osteoporosis. Recently Fang et al. [41] identified 62 polymorphisms in potentially functional areas of the VDR gene and they demonstrated that the polymorphisms in the 5' promoter region and the 3'UTR of VDR contribute to the fracture risk in a large population. Osteoporosis is a complex disease with multifactorial aetiology and therefore, a minor contributing pathogenetic role of the VDR gene polymorphisms in specific cases, and in combination with other risk factors (such as dietary intake and exogenous hormones) that modulate the development of osteoporosis, cannot be totally excluded. Therefore, the relationship between the VDR polymorphisms and osteoporosis remains an unresolved issue and case-control studies that investigate gene-environment interaction might elucidate further genetics of osteoporosis.

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