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BRIEF ARTICLE

Vitamin D receptor gene polymorphisms and colorectal cancer risk: A systematic meta-analysis

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Abstract

AIM: To investigate the relationship between polymorphisms present in the vitamin D receptor (*VDR*) gene and colorectal cancer risk, a systematic meta-analysis of population-based studies was performed.

METHODS: A total of 38 relevant reports published between January 1990 and August 2010 were identified, of which only 23 qualified for this meta-analysis based on our selection criteria. Five polymorphic variants of the *VDR* gene, including *Cdx*-2 (intron 1e) and *Fok*I (exon 2) present in the 5′ region of the gene, and *Bsm*I (intron 8), *Apa*I (intron 8), and *Taq*I (exon 9) sites present in the 3′ untranslated region (UTR), were evaluated for possible associations with colorectal cancer risk. Review manager 4.2 was used to perform statistical analyses.

RESULTS: In the meta-analysis performed, only the *Bsm*I polymorphism was found to be associated with colorectal cancer risk. In particular, the *Bsm*I B genotype was found to be related to an overall decrease in the risk for colorectal cancer [*BB vs bb*: odds ratio (OR) = 0.87, 95% CI: 0.80-0.94, $P = 3 \times 10^{-4}$; *BB vs Bb* + *bb*: OR = 0.90, 95% CI: 0.84-0.97, $P = 5 \times 10^{-4}$]. Moreover, in subgroup analyses, the *Bsm*I *B* genotype was significantly associated with colon cancer, and not rectal cancer. An absence of between-study heterogeneity was also observed.

CONCLUSION: A meta-analysis of 23 published studies identified the *Bsm*I polymorphism of the *VDR* gene to be associated with an increased risk of colon cancer.

Key words: Vitamin D receptor; Polymorphism; Meta-analysis; Colorectal cancer

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INTRODUCTION

Colorectal cancer represents the third most common cancer worldwide, second only to lung cancer and gastric

cancer[1]. Furthermore, it is estimated that there are more than 370 000 cases of colon and rectal cancer diagnosed in Europe every year, with 200 000 cases resulting in death[2]. However, the underlying etiology of colorectal cancer, including cancerous growths of the colon, rectum, and appendix, remains poorly understood. It has been proposed that some categories of external agents, including physical, chemical, and biological carcinogens, may contribute to the development of this disease, and the role of these factors in carcinogenesis would depend largely on genetic factors. Correspondingly, a recent study showed that insufficient levels of vitamin D may result in colorectal cancer[3]. Furthermore, genetic variations in genes controlling vitamin D activity would be hypothesized to play an important role in determining susceptibility to colorectal cancer.

In vivo, vitamin D helps bones and muscles grow, and may also help prevent many diseases, such as prostate cancer and breast cancer. The biological activity of vitamin D is mediated by the vitamin D receptor (*VDR*)[4], which interacts with other cell signaling pathways to influence cell behavior. Expression of *VDR* has been detected in various organs and tissues of the human body, including the kidney and bone cells. *VDR* is also expressed in normal colon mucosa[5]. In the intestine, *VDR* plays an important role in regulating cell proliferation, differentiation, and the induction of apoptosis[6]. Furthermore, *VDR* may be associated with the effects of calcium on colorectal epithelial proliferation[7].

Molecular epidemiological studies have shown that polymorphisms in the *VDR* gene may be linked to biological functions of vitamin D. At the 5' end of the *VDR* gene, a *Fok*I polymorphism (rs2228570/rs10735810, exon 2) has been associated with a frameshift in the *VDR* protein[8]. Moreover, polymorphisms in the 3' untranslated region (UTR), including *Bsm*I (rs1544410, intron 8), *Apa*I (rs7975232, intron 9), and *Taq*I (rs731236, exon 9) sites, have been shown to influence gene transcription and mRNA stability[9]. Additionally, these polymorphisms have exhibited the potential for strong linkage disequilibrium (LD)[10,11], and functional differences have been associated with the associated haplotypes[11,12]. Given that polymorphisms in the *VDR* gene could potentially influence the binding of 1, 25$(OH)_2D_3$ and the anti-proliferative effects of vitamin D, *VDR* polymorphisms have been hypothesized to be associated with colorectal cancer risk.

In 2001, the first report of an association between colorectal cancer and the *VDR* gene was published by Kim and colleagues[13]. They identified a random subset of 393 cases of colorectal adenomas and 406 colonoscopy-negative controls from a clinic-based, case-control study conducted in the United States between 1991 and 1994. Based on their analysis, the *Bsm*I *BB* genotype was found to be associated with a reduced risk of colorectal adenoma when intake of calcium and vitamin D was reduced. In addition to the *Bsm*I site[12,14-23]. Other polymorphic sites present in the *VDR* gene, including *Cdx*-2[12,19,21,24,25], *Fok*I[12,15,16,18,19,21-24,26-33], *Apa*I[12,16,19,21,34], and *Taq*I[12,16,19,23,24,29,31,33-35], have been evaluated in genetic association studies. However, the results are inconsistent. Since it can be difficult for individual studies to achieve sufficient statistical power to detect associations between *VDR* polymorphisms and colorectal cancer risk, a meta-analysis that combines data from all published studies may detect genetic associations more accurately. In addition, a reduced probability of false-negatives might also be achieved[36]. Therefore, a systematic meta-analysis of population-based studies was performed to investigate the association between *VDR* polymorphisms and the risk of colorectal cancer. Based on the search strategy and criteria used, 23 studies were analyzed which identified several important polymorphic variants.

MATERIALS AND METHODS

Search strategy and data extraction

To examine the association between *VDR* polymorphisms and colorectal cancer risk, a search of the MEDLINE database (from January 1990 to August 2010) and the US National Library of Medicine's PubMed database (http://www.ncbi.nlm.nih.gov/pubmed) was performed. In addition, various scientific research tools available on the web were used to search relevant references such as Google (http://scholar.google.com/) and Scirus (http://www.scirus.com/). In particular, data relevant to five well-characterized polymorphic variants was identified, including: *Cdx*-2, *Fok*I, *Bsm*I, *Apa*I, and *Taq*I sites within *VDR*. Keywords used in searches included "vitamin D receptor" in combination with "polymorphism", "vitamin D", "genotype", "allele", "colorectal cancer", or "risk".

Papers selected for this meta-analysis included a case-control study and complete data, including the authors' names; the subjects' region/country; year of publication; numbers of cases and controls; mean age (or range) of the case/control group; diagnostic criteria used; and number of subjects with the *VDR* genotype in both case and control groups. All relevant references that met these inclusive criteria and that were published as articles or abstract containing original data, were included in this study. In contrast, case-only studies, studies with incomplete data, or studies with inadequate control groups were excluded. In addition, the data extracted needed to conform to the guidelines of MOOSE, a proposal for reporting meta-analyses of observational studies[37]. If the same or overlapping data were reported in multiple publications, the most recent publication was selected[38].

Statistical analysis

For each data set included in this study, the odds ratios (ORs) and corresponding 95% CI for the incidence of cancer in subjects with or without particular restriction sites (lowercase *vs* uppercase lettering), was compared. Furthermore, deviations from the Hardy-Weinberg equilibrium for each control group were assessed using the goodness-of-fit test. To estimate associations with colorectal cancer risk, various genotypic models were

Table 1 Characteristics of case-control studies included in the meta-analysis

First author	Year	Country	Racial descent	Mean age in cases	Mean age in controls	Cases/ controls	Genotyping method	Quality control	Adjusted	Studied polymorphisms
Ingles *et al*[11]	1998	United States	American	62.3	62.2	373/394	PCR-RFLP	Yes	Yes	*Fok*I
Kim *et al*[13]	2001	United States	American	58.0 ± 9.7	53.0 ± 11.0	393/406	TaqMan	Yes	Yes	*Bsm*I
Peters *et al*[27]	2001	United States	American	18-74	18-74	208/184	PCR-RFLP	Yes	NR	*Fok*I
Slatter *et al*[23]	2001	United States	American	NR	NR	424/366	PCR-RFLP	NR	Yes	*Fok*I, *Bsm*I, *Taq*I
Speer *et al*[14]	2001	Hungary	European	64	63	56/112	PCR-RFLP	NR	NR	*Bsm*I
Grau *et al*[29]	2003	United States	American	60.8 ± 9.0	60.9 ± 9.0	372/379	PCR-RFLP	Yes	Yes	*Fok*I, *Taq*I
Wong *et al*[28]	2003	Singapore	Asian	66	56.5	217/890	PCR-RFLP	Yes	Yes	*Fok*I
Peters *et al*[35]	2004	United States	American	62.9	62.3	763/774	PCR-RFLP	Yes	NR	*Taq*I
Slattery *et al*[15]	2004	United States	American	30-79	30-79	1936/2130	PCR-RFLP	NR	Yes	*Fok*I, *Bsm*I
Murtaugh *et al*[30]	2006	United States	American	30-79	30-79	2450/2821	PCR-RFLP	NR	Yes	*Fok*I
Park *et al*[16]	2006	South Korea	Asian	55	55	190/354	PCR-RFLP	NR	Yes	*Fok*I, *Bsm*I, *Apa*I, *Taq*I
Flügge *et al*[12]	2007	Germany	European	61.9 ± 10.0	62.2 ± 11.2	256/256	PCR-RFLP	Yes	Yes	*Cdx*-2, *Fok*I, *Bsm*I, *Apa*I, *Taq*I
Kadiyska *et al*[19]	2007	Bulgaria	European	59	59 ± 5	133/94	PCR-RFLP	NR	Yes	*Bsm*I
Slattery *et al*[18]	2007	United States	American	30-79	30-79	2380/2990	TaqMan	Yes	Yes	*Fok*I, *Bsm*I
Yaylim-Eraltan *et al*[31]	2007	Turkey	European	59.1 ± 4.0	52.0 ± 0.8	26/52	PCR-RFLP	NR	Yes	*Fok*I, *Taq*I
Grünhage *et al*[32]	2008	Germany	European	65 ± 9	63 ± 8	192/220	PCR-RFLP	NR	Yes	*Fok*I
Hubner *et al*[17]	2008	United Kingdom	European	NR	NR	137/409	TaqMan	Yes	Yes	*Cdx*-2, *Fok*I, *Bsm*I, *Apa*I, *Taq*I
Ochs-Balcom *et al*[24]	2008	United States	American	62.8 ± 10.2	58.5 ± 12.1	250/246	TaqMan	Yes	Yes	*Cdx*-2, *Fok*I, *Taq*I
Parisi *et al*[20]	2008	Spain	European	NR	NR	170/120	PCR-RFLP	NR	Yes	*Bsm*I
Theodoratou *et al*[21]	2008	United Kingdom	European	62.0 ± 10.8	62.4 ± 10.5	3005/3072	Microarray	Yes	Yes	*Cdx*-2, *Fok*I, *Bsm*I, *Apa*I
Wang *et al*[33]	2008	China	Asian	38-78	19.6 ± 1.3	69/218	PCR-RFLP	NR	Yes	*Fok*I
Jenab *et al*[22]	2009	Europe	European	NR	NR	1248/1248	TaqMan	Yes	Yes	*Fok*I, *Bsm*I
Mahmoudi *et al*[34]	2010	Iran	Asian	52.7 ± 14.0	44.4 ± 17.7	160/180	PCR-RFLP	Yes	Yes	*Apa*I, *Taq*I

NR: Not reported.

selected, including codominant, additive, recessive, and dominant. Both the Peto Mantel-Haenszel fixed-effects model and the DerSimonian Laird random-effects model (with weights based on the inverse variance) were used to calculate summary ORs, and both within- and between-study variations were considered[39]. A *P*-value less than 0.10 was considered statistically significant when comparing trials showing heterogeneity, and random-effects analysis was selected. In contrast, fixed-effects analysis was used for comparing trials exhibiting homogeneity. Inverted funnel plots were also used to examine asymmetry, in which the ORs were plotted on a logarithmic scale against the inverse of their corresponding standard errors[40]. In the presence of publication bias, the funnel plot was asymmetric and the data showed remarkable skewness. There may be many reasons for this, most notably that some studies with negative findings are not published. In contrast, the plots were symmetric when bias was absent.

All statistical analyses were performed using the Statistical Package for Social Sciences (SPSS, version 13.0) and Review Manager (version 4.2, The Cochrane Collaboration), and all *P*-values were two-sided.

RESULTS

Characteristics of case-control studies included in the meta-analysis

According to the criteria defined above, 38 published studies relevant to the *VDR* gene and colorectal cancer risk were reviewed. Fifteen of these papers were excluded due to insufficient clarity in data presentation, repeated literature, or significant differences were present in their study design compared with the other papers identified[41]. The remaining 23 eligible case-control studies are listed in Table 1, and were included in a meta-analysis to investigate possible associations between *Cdx*-2, *Fok*I, *Bsm*I, *Apa*I, and *Taq*I polymorphisms present in the *VDR* gene and the risk of colorectal cancer.

In 21/23 studies, data regarding the 5' end of the *VDR* gene were provided. In four of these studies, 2639 cases and 2948 controls were analyzed for the *Cdx*-2 polymorphism, while 17 studies included 13 301 cases and 15 942 controls analyzed for the *Fok*I polymorphism. In addition, the 3' UTR region of the *VDR* gene has been analyzed. For example, 12 studies containing 10 083 cases and 11 242 controls analyzed the *Bsm*I polymorphism, 5 studies including 2739 cases and 3200 controls analyzed the *Apa*I polymorphism, and 9 studies including 2580 cases and 3016 controls analyzed the *Taq*I polymorphism.

Among controls, the frequency of the *c* allele at the *Cdx*-2 site ranged from 65.6% in Berlin-Bush populations of Germany, to 80.0% in a United Kingdom population[12,19,21,24,25]. In contrast, the frequency of the *f* allele of *Fok*I among controls ranged from 31.7% in Turkey, to 47.2% in a Singapore population[12,15,16,18,19,21-24,26-33]. The frequency of the *b* allele at *Bsm*I among controls ranged from 56.1% in a Bulgarian population, to 94.7% in a Korean population[12,14-23], while the frequency of the *a* allele at *Apa*I

Table 2 Summary odds ratios and 95% CI in the vitamin D receptor gene

SNP	Model	Total No. cases	Total No. controls	OR (95% CI)[1]	*P* value[2]	*P* value[3]
Cdx-2	Codominant (*CC vs cc*)	152/1561	146/1820	1.25 (0.98-1.59)	0.07	0.26
	Codominant (*Cc vs cc*)	926/2487	982/2802	1.09 (0.97-1.22)	0.15	0.64
	Codominant (*C vs C*)	1230/4048	1279/4622	1.10 (1.01-1.21)	0.03	0.47
	Dominant (*CC + Cc vs cc*)	1078/1561	1208/1820	0.98 (0.88-1.09)	0.72	< 0.001
	Recessive (*CC vs Cc + cc*)	152/2487	146/2802	1.22 (0.96-1.54)	0.10	0.23
*Fok*I	Codominant (*ff vs FF*)	1844/5068	2377/5982	0.94 (0.87-1.01)	0.09	0.001
	Codominant (*Ff vs FF*)	6189/11 257	7583/13 565	0.98 (0.93-1.03)	0.34	0.001
	Codominant (*f vs F*)	9867/16 320	12 190/19 329	0.97 (0.94-1.00)	0.07	< 0.001
	Dominant (*ff + Ff vs FF*)	8033/5068	9960/5982	0.96 (0.92-1.01)	0.15	< 0.001
	Recessive (*ff vs FF + Ff*)	1844/11 257	2377/13 565	0.95 (0.89-1.02)	0.13	0.01
*Bsm*I	Codominant (*BB vs bb*)	1512/3838	1817/4122	0.87 (0.80-0.94)	< 0.001	0.65
	Codominant (*Bb vs bb*)	4733/8571	5303/9425	0.94 (0.88-0.99)	0.03	0.64
	Codominant (*B vs b*)	7757/12 409	8937/13 577	0.93 (0.90-0.97)	< 0.001	0.85
	Dominant (*BB + Bb vs bb*)	6245/3838	7120/4122	0.92 (0.87-0.97)	0.003	0.84
	Recessive (*BB vs Bb + bb*)	1512/8571	1817/9425	0.90 (0.84-0.97)	0.006	0.28
*Apa*I	Codominant (*AA vs aa*)	748/578	1004/603	0.85 (0.73-0.99)	0.03	0.06
	Codominant (*Aa vs aa*)	1378/1956	1593/2196	0.91 (0.79-1.04)	0.18	0.39
	Codominant (*A vs a*)	2944/2534	3601/2799	0.92 (0.85-0.99)	0.02	0.04
	Dominant (*AA + Aa vs Aa*)	2161/578	2597/603	0.89 (0.78-1.01)	0.07	0.12
	Recessive (*AA vs Aa + aa*)	783/1956	1004/2196	0.89 (0.80-1.00)	0.05	0.21
*Taq*I	Codominant (*tt vs TT*)	382/1112	398/1320	1.05 (0.89-1.24)	0.58	0.07
	Codominant (*Tt vs TT*)	1086/2198	1298/2618	0.93 (0.83-1.05)	0.23	0.30
	Codominant (*t vs T*)	1850/3310	2098/3938	0.99 (0.92-1.08)	0.86	0.10
	Dominant (*tt + Tt vs TT*)	1468/1112	1696/1320	0.95 (0.85-1.07)	0.42	0.37
	Recessive (*tt vs Tt + TT*)	382/2198	398/2618	1.07 (0.92-1.25)	0.38	0.01

[1]Based on fixed effects model; [2]Test for overall effect; [3]Test for heterogeneity. OR: Odds ratios.

among controls ranged from 23.0% in a Korean population to 49.3% in a population of the United Kingdom[12,16,19,21,34]. Lastly, the frequency of the *t* allele at *Taq*I among controls ranged from 8.8% in a Korean population to 43.6% in a population of the United States[12,16,19,23,24,29,31,33-35].

Qualitative assessment of included studies

Genotyping of the *Cdx*-2, *Fok*I, *Bsm*I, *Apa*I and *Taq*I polymorphisms was performed using the polymerase chain reaction-restriction fragment length polymorphism technique in 75% of the studies included in this meta-analysis. Due to the low sensitivity of this classic technology, quality control of this genotyping was required, and included blindness to the case-control status, random repeats of samples, or validation using a different genotyping method. However, only 38.9% (7/18) of the eligible studies provided sufficient quality control. Regarding sample size, only 5/24 (20.8%) studies employed more than 1000 cases or controls. Moreover, most of these studies were associated with poor statistical power due to sample sizes that were less than 500 and in some cases contained less than 100 cases or controls.

Assessment of Hardy-Weinberg proportion is regarded as an important criterion for evaluating genetic association studies[38]. Most of the studies included in this meta-analysis reported genotype frequencies in their control groups that were consistent with Hardy-Weinberg proportions ($P > 0.05$). For example, deviations from Hardy-Weinberg proportions in controls were observed in three studies for *Fok*I[29,31,32], two studies of *Bsm*I[19,21], one study of *Apa*I[19], and two studies of *Taq*I[23,24].

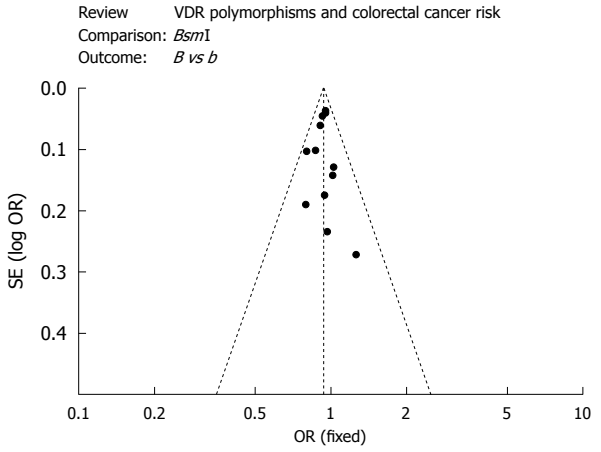

Figure 1 A funnel plot was used to estimate the publication bias of the studies included in the meta-analysis performed.

Funnel plotting was performed to evaluate whether publication bias was present in the meta-analysis performed. As shown in Figure 1, the shapes of the funnel plots obtained appear to be symmetrical in codominant, dominant, and recessive models, suggesting that publication bias is absent in the meta-analysis performed.

Cdx-2, FokI, BsmI, ApaI, TaqI polymorphisms and colorectal cancer risk

A heterogeneity test of potential associations between the *Cdx*-2, *Fok*I, *Bsm*I, *Apa*I, and *Taq*I polymorphisms and risk of colorectal cancer are presented in Tables 2 and 3.

Table 3 The *Bsm*I effect odds ratios stratified by anatomical site

Model	Colon cancer			Rectal cancer		
	Total No. cases/controls	OR (95% CI)	*P* value[1]	Total No. cases/controls	OR (95% CI)	*P* value[1]
Codominant (*BB vs bb*)	1365/1581	0.80 (0.68-0.93)	0.004/0.21	659/790	0.92 (0.73-1.15)	0.46/0.90
Codominant (*Bb vs bb*)	2257/2438	0.99 (0.88-1.12)	0.92/0.95	1029/1240	0.94 (0.80-1.11)	0.48/0.21
Codominant (*B vs b*)	5328/5972	0.91 (0.84-0.98)	0.01/0.25	2440/2966	0.95 (0.85-1.06)	0.38/0.99
Dominant (*BB + Bb vs bb*)	2664/2986	0.94 (0.84-1.05)	0.25/0.65	1220/1483	0.94 (0.77-1.16)	0.59/0.40
Recessive (*BB vs Bb + bb*)	2664/2986	0.80 (0.69-0.92)	0.002/0.19	1220/1483	0.93 (0.80-1.09)	0.40/0.51

[1]Test for overall effect and heterogeneity, respectively. OR: Odds ratios.

***Cdx*-2 polymorphism:** Currently, only four studies have investigated the relationship between the *VDR Cdx*-2 polymorphism and colorectal cancer risk, and all of these studies were in Hardy-Weinberg equilibrium[12,19,21,24,25]. Furthermore, in the overall and subgroup analyses performed, the *Cdx*-2 polymorphism did not appear to be linked to colorectal cancer risk.

***Fok*I polymorphism:** Seventeen studies included in the meta-analysis performed found the *Fok*I polymorphism to be statistically heterogeneous in all genetic models ($P \leqslant 0.01$)[12,15,16,18,19,21-24,26-33]. Moreover, no significant association was found between *Fok*I and colorectal cancer risk in overall and subgroup analyses.

***Bsm*I polymorphism:** A total of 12 studies examined the association between colorectal cancer and the *Bsm*I polymorphism, and there was little statistical evidence of heterogeneity among the studies ($P \geqslant 0.28$)[12,14-23]. Individuals with the *BB* genotype (OR = 0.87; 95% CI = 0.80-0.94, $P = 3 \times 10^{-4}$; $P = 0.65$ for heterogeneity), or the *Bb* genotype (OR = 0.94; 95% CI: 0.88-0.99, $P = 0.03$; $P = 0.48$ for heterogeneity), were associated with a significant decrease in colorectal cancer risk compared with patients carrying the *bb* genotype. The Dominant model (*BB + Bb vs bb*) and the recessive model (*BB vs Bb + bb*) also showed a significant association with colorectal cancer risk, with the associated ORs being 0.92 (95% CI: 0.87-0.97, $P = 0.003$; $P = 0.84$ for heterogeneity) and 0.90 (95% CI: 0.84-0.97, $P = 0.006$; $P = 0.28$ for heterogeneity), respectively (Figure 2). Although two of these studies were not consistent with Hardy-Weinberg proportions[17,21], the effect was negligible. In addition, the *BB* genotype showed a decreased risk for colon cancer compared with the *bb* (OR = 0.80, 95% CI: 0.68-0.93, $P = 0.004$; $P = 0.21$ for heterogeneity), or *Bb + bb* genotypes (OR = 0.80, 95% CI: 0.69-0.92, $P = 0.002$; $P = 0.19$ for heterogeneity). However, no significant differences were observed between these polymorphisms and rectal cancer risk (Table 3).

***Apa*I polymorphism:** The association between the *Apa*I polymorphism and colorectal cancer was investigated in five studies, of which only one study was not consistent with Hardy-Weinberg proportions[12,16,19,21,34]. Moreover, although the codominant model (*AA vs aa*, OR = 0.83; 95% CI: 0.71-0.97, $P = 0.02$) showed a significant association with colorectal cancer risk, the *P* value of 0.05 suggested that this genetic model was statistically heterogeneous.

*Taq*I polymorphism. Except for the recessive model (*tt vs Tt + TT*, $P = 0.06$), there was little evidence of statistical heterogeneity among the nine studies that investigated an association between the *Taq*I polymorphism and colorectal cancer risk ($P \geqslant 0.35$)[12,16,19,23,24,29,31,33-35]. When the two studies in which controls were not in Hardy-Weinberg equilibrium were excluded, the pooled ORs for all genetic models for *Taq*I were shifted, yet the results remained null[23,24].

DISCUSSION

This study was undertaken to assess whether *VDR* polymorphisms in both the 5' (*Cdx-2* and *Fok*I) and 3' (*Bsm*I, *Apa*I and *Taq*I) regions of the *VDR* gene are associated with colorectal cancer risk. A total of 38 reports had previously evaluated a possible genetic association, and only 23 of these were eligible for this study based on the selection criteria employed. The pooled ORs (95% CI) for these studies were identical according to both fixed- and random-effects models. Moreover, only the polymorphic variant, *Bsm*I, was found to be associated with increased risk for colorectal cancer. The meta-analysis performed also showed that the *Bsm*I *B* genotype was related to a significant decrease in overall risk for colorectal cancer, with the co-dominant *BB* and the *BB + Bb vs bb* dominant model exhibiting 0.87- and 0.92-fold increases in the risk for disease, respectively. The *BB vs Bb + bb* recessive model also had a 90% decreased risk for colorectal cancer.

In addition, subgroup analyses by anatomical site identified the *Bsm*I polymorphism to be significantly associated with colon cancer, and not rectal cancer. Furthermore, compared with the *bb* or *Bb + bb* genotypes, the *BB* genotype was associated with a 90% decrease in the risk for colon cancer. However, it remains unclear why the *Bsm*I site is related to the risk of colon cancer, and not rectal cancer. It is possible that the difference in epithelial cells between the two cancers plays a role, with ciliated columnar epithelial cells being present in the lining of the colon, while squamous epithelial cells are present in the rectum. The role of the micro-environment may also be a contributing factor, since physical damage as a result of oxygen or poisonous food residues is more likely to influence the rectum than the colon. In addition,

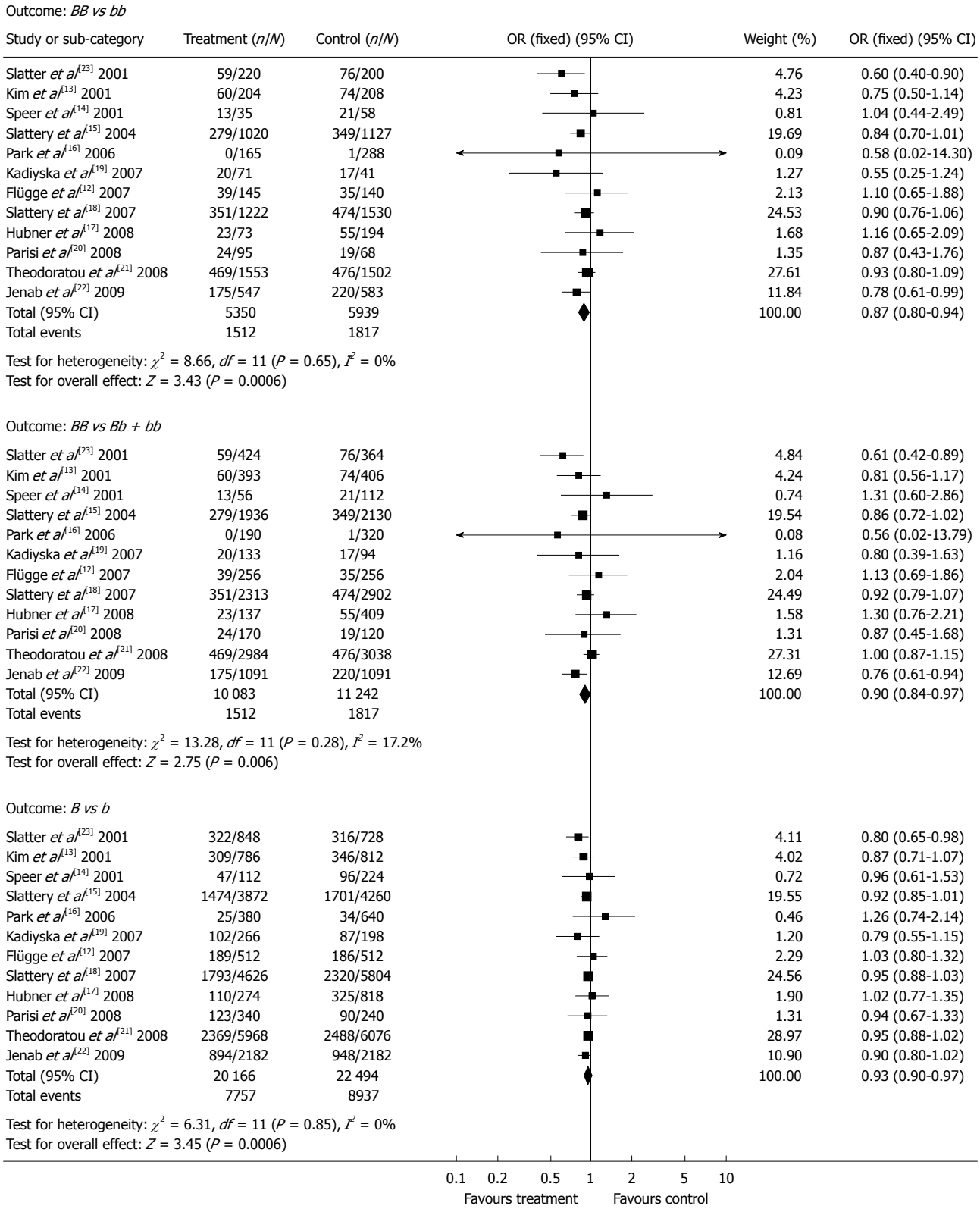

Figure 2 Forest plot of the meta-analysis performed to investigate the association between the *BsmI* polymorphism of the vitamin D receptor gene and colorectal cancer risk (fixed-effects model).

genetic factors may have a more important role in colon cancer than rectal cancer.

Based on the distribution differences observed between cases of colorectal cancer and controls, we hypothesized that the *Bsm*I *B* allele might have a protective effect against tumorigenesis. Correspondingly, of the 12 relevant reports reviewed, 9 studies supported this hypothesis. In these studies, the populations analyzed were from Asia (n = 1), Europe (n = 4), and the United States (n = 4). In addition, Jenab *et al*[22] evaluated different Cau-

casian populations from 23 centers in Denmark, France, Greece, Germany, Italy, the Netherlands, Norway, Spain, Sweden, and the United Kingdom. In this study, the *BB* genotype, rather than the wild-type *bb* genotype, was associated with a reduced risk of colorectal cancer. The *Bsm*I *BB* genotype was also found to be associated with a reduced risk of colorectal cancer among non-aspirin/NSAID users[42]. Thus, multiple lines of evidence support the hypothesis that the *Bsm*I *B* allele mediates a protective effect against the development of cancers in the digestive tract, especially colon cancer.

The *Bsm*I polymorphism is located in the 3' UTR of the *VDR* gene, and does not alter the amino acid sequence of the *VDR* protein. Thus, for a single *Bsm*I polymorphism, there is a low probability that it directly influences *VDR* function[9]. The *Bsm*I polymorphism also does not affect mRNA or protein levels of *VDR*[20], or levels of $25(OH)_2D_3$ or $125(OH)_2D_3$[43]. However, the *Bsm*I site does exhibit strong LD with other *VDR* polymorphisms, including e*Tru9*I, *Apa*I, *Taq*I, and *Poly(A)* microsatellites. Based on these results, it appears that the *Bsm*I polymorphism affects some type of biological function, and these could potentially include regulation of *VDR* transcription, translation, or RNA processing[9]. Other unidentified SNPs in the *VDR* gene, as well as SNPs in other genes such as CYP3A5[44], may also affect the function of the *Bsm*I. Furthermore, patients carrying the *Bsm*I allele have also been shown to have significantly higher levels of *erbB-2* expression, suggesting other tumor-related molecules may also be involved in the function of the *Bsm*I polymorphism[14].

Although the *Bsm*I *B* allele has been associated with a protective effect, the frequency of this effect has been found to be lower in Asian populations than in Caucasian populations. However, this is inconsistent with the incidence of colorectal cancer identified in recent epidemiological data[2]. Moreover, in the meta-analysis performed in the present study, no significant association was found between *VDR* genotypes and the risk of colorectal cancer in group analyses (not shown). In combination, these results suggest that other factors may be involved. For example, environment, food, and lifestyle may play a more significant role, in combination with genetic factors, in the occurrence and development of colorectal cancer than previously thought, which would potentially account for the inconsistent results obtained from previous studies.

COMMENTS

Background

Colorectal cancer is one of the most common cancers worldwide, and its incidence is increasing with each year. However, the underlying etiology of colorectal cancer remains unclear. Several epidemiologic studies have reported that 1, $25(OH)_2D_3$ can reduce the risk of colorectal cancer, and thus, vitamin D receptor (*VDR*), a crucial mediator of the cellular effects of 1, $25(OH)_2D_3$, may play an important role in the occurrence and development of colorectal cancer.

Research frontiers

Recently, several polymorphic variants of the *VDR* gene have been reported to be associated with the risk of colorectal cancer. However, the published findings remain inconsistent. In this study, the authors conducted a systematic meta-analysis to evaluate the evidence regarding this association.

Innovations and breakthroughs

In the present study, all relevant reports published between January 1990 and August 2010 were reviewed, with a focus on five well-characterized polymorphic variants of *VDR*: *Cdx*-2, *Fok*I, *Bsm*I, *Apa*I, and *Taq*I. In the meta-analysis performed, *Bsm*I was found to be associated with colorectal cancer, while the *Cdx*-2, *Fok*I, *Apa*I, and *Taq*I sites did not exhibit any significant association. Moreover, the *Bsm*I '*B*' genotype was associated with a significant decrease in the risk of colorectal cancer, especially colon cancer. Based on these results, it is hypothesized that *Bsm*I may mediate a protective effect on tumorigenesis.

Applications

The results of this meta-analysis have the potential to partly explain the genetics that influence the pathogenesis of colorectal cancer. This study also helps provide a basis for clinical diagnosis and methods for early intervention.

Peer review

The authors performed a systematic meta-analysis of population-based studies to investigate the association between *VDR* polymorphisms and colorectal cancer risk. The authors found a *Bsm*I site in the 3' UTR of the *VDR* gene to be associated with colon cancer risk, and then analyzed the underlying mechanism. The results are interesting and may help explain the genetic mechanism of colorectal carcinogenesis.

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