RESEARCH ARTICLE

Vitamin D Receptor BsmI Polymorphism and Colorectal Cancer Risk: an Updated Analysis

Kun Yu[1&], Jing Yang[2&], Yan Jiang[3], Run Song[4], Qing Lu[1]*

Abstract

    <u>Background</u>: Previous studies have investigated the association between the vitamin D receptor (VDR) BsmI polymorphism and colorectal cancer (CRC) susceptibility, but the results were conflicting. The aim of this study is to quantitatively summarize the relationship between this polymorphism and CRC risk. <u>Materials and Methods</u>: Two investigators independently searched the Medline, Embase, China National Knowledge Infrastructure (CNKI) and Chinese Biomedicine databases for studies published before November 2013. Summary odds ratios (ORs) and 95% confidence intervals (95%CIs) for VDR BsmI polymorphism and CRC were calculated in a fixed-effects model (the Mantel-Haenszel method) and a random-effects model (the DerSimonian and Laird method) when appropriate. <u>Results</u>: This meta-analysis included 14 case-control studies, which included 10,822 CRC cases and 11,779 controls. Overall, the variant genotype (BB) of the BsmI was associated with a lower CRC risk when compared with the wild-type bb homozygote (OR=0.66, 95%CI: 0.49-0.88). Similarly, a decreased CRC risk was also found in the dominant and recessive models. When stratifying for ethnicity, source of controls, and study sample size, associations were observed among Caucasians, population-based studies and studies with large study sample size (>1000 subjects). Limiting the analysis to the studies within Hardy-Weinberg equilibrium, the results were persistent and robust. No publication bias was found in the present study. <u>Conclusions</u>: This updated meta-analysis suggests that the VDR BsmI polymorphism may be associated with a moderate protective effect against CRC.

Keywords: Colorectal cancer - polymorphism - VDR - meta-analysis

Introduction

    Colorectal cancer (CRC) is the third most commonly diagnosed cancer in males and the second in females, with over 1.2 million new cancer cases and 608,700 deaths estimated to have occurred in 2008 (Jemal et al., 2011). In Asia, CRC is the fourth leading cause of mortality by cancer, and its incidence is increasing (Sung et al., 2005). In recent years, the incidence of CRC is increasing in China, which accounts for about 6.5% of total cancers in urban areas and 4.6% in rural areas (Zhao et al., 2010). Many factors contribute to the development of CRC, including environmental factors; for example, Garland and Garland (Garland and Garland, 1980) hypothesized two decades ago that higher incidence rates for CRC in areas with lower sunlight exposure might be attributable to lower levels of vitamin D. But not only environmental factors are involved. In recent years, the relationship between the genetic susceptibility to CRC and single nucleotide polymorphism (SNP) has become a focus of research.

    Vitamin D is an important factor for calcium homeostasis and modulation of cell cycle kinetics and is thought to protect against colorectal cancer (CRC) in sufficient concentrations (Giovannucci, 2007; Garland et al., 2009; Pilz et al., 2009; van der Rhee et al., 2009). Many of the actions of vitamin D are thought to be mediated by the vitamin D receptor (VDR), a member of the nuclear receptor super-family that is present in different cell types. The VDR can also regulate other vitamin D inducible genes involved in processes of inflammation, immune function, estrogen metabolism, insulin-like growth factor-I signaling, and regulation of intestinal calcium absorption (Jones et al., 1998; Uitterlinden et al., 2004; Thorne and Campbell, 2008). The importance of the VDR in relation to cancer risk has been experimentally demonstrated in vitro and in vivo (Thorne and Campbell, 2008). It is possible that the effects of vitamin D may differ among individuals depending on variations in the activity of the VDR.

    The human VDR gene, which consists of nine exons, is located on chromosome 12. More than 100 single nucleotide polymorphisms (SNPs) have been described

*[1]Institute of Stem Cell and Developmental Biology, West China Second Hospital, Sichuan University, [4]Chengdu BioSciTec Biotechnology Co., Ltd, Chengdu, [2]Department of Microbiology, Hubei University of Medicine, Shiyan, [3]Department of Laboratory Animal Center, Guiyang Medical College, Guiyang, China [&]Equal contributors *For correspondence: hxluqing@163.com*

within its 67076-bp sequence. Molecular epidemiological studies have shown that polymorphisms in the VDR gene may be linked to biological functions of vitamin D. Such as, the VDR BsmI polymorphism is located in intron 8 of the VDR gene (Kim et al., 2001). It is thought that the 3-UTR region of the VDR gene is involved in the regulation of mRNA stability, so polymorphisms in this region could be involved in the degradation of the VDR mRNA and consequently in receptor density (Beelman and Parker,1995). Given the importance of the gene, a number of studies have explored the role of VDR polymorphisms in modulating cancer risk in various tissues.

Over the last two decades, a number of case-control studies were conducted to investigate the association between VDR BsmI polymorphism and CRC risk in humans. However, the results of these studies are conflicting. In recent years, two meta-analyses (Touvier et al., 2011; Bai et al., 2012) had been published to assess the association between VDR BsmI polymorphism and CRC risk. The two studies found that this polymorphism was associated with a decreased CRC risk and suggested that further studies were needed to confirm these results. However, the two studies had some limitations, such as relatively small sample size, few included studies were from Asians, some of the extracted data (revealed in Bai et al's study (Bai et al., 2012)) were incorrect. Moreover, a number of studies were published after that period. In order to derive a more comprehensive estimation of the association between VDR BsmI polymorphism and CRC risk, we conducted a meta-analysis from 14 eligible case-control studies to evaluate the association.

Materials and Methods

Literature search strategy

We searched the Medline, Embase, China National Knowledge Infrastructure (CNKI) and Chinese Biomedicine databases for all articles on the association between VDR polymorphisms and CRC risk (last search update 21th November 2013). The following key words were used: "colorectal" or "colo*", "cancer" or "tumor" or "carcinoma", "VDR" or "Vitamin D Receptor", and "polymorphism" or "allele" or "genotype". The search was without restriction on language, conducted on human subject. The reference lists of reviews and retrieved articles were hand searched at the same time.

Inclusion and exclusion criteria

The following criteria were used to include published studies: (i) case-control studies were conducted to evaluate the association between VDR BsmI polymorphism and CRC risk; (ii) sufficient genotype data were presented to calculate the odds ratios (ORs) and 95% confidence intervals (95%CIs); (iii) The paper should clearly describe CRC diagnoses and the sources of cases and controls. Major reasons for exclusion of studies were (i) review, or meta-analysis, or editorial, or comment; (ii) duplicated studies, or studies without raw data we need; (iii) studies that focused on HNPCC or FAP. Family-based studies of pedigrees with several affected cases per family were also excluded, because their analysis is based on linkage considerations.

If more than one article was published by the same author using the same case series, we selected the study where the most individuals were investigated.

Data extraction

Two investigators (Kun Yu and Jing Yang) extracted information from all eligible publications independently according to the inclusion criteria listed above. Disagreements were resolved by discussion with co-authors. The following characteristics were collected from each study: name of first author, year of publication, ethnicity, the country of participants, number of cases and controls, genotyping methods, minor allele frequency (MAF) in controls, evidence of Hardy-Weinberg equilibrium (HWE) in control group, source of control group (population- or hospital-based controls), genotypes and others (Table 1). If original genotype frequency data were unavailable in relevant articles, a request was sent to the corresponding author for additional data. In this study, population-based case-control study (PCC) was defined as controls from healthy people, and hospital-based case-control study (HCC) were from hospitalized patients and outpatients.

Statistical analysis

We first assessed HWE in the controls for each study using goodness-of-fit test (chi-square or Fisher's exact test) and a $P<0.05$ was considered as significant disequilibrium. The strength of the association between CRC and the VDR BsmI polymorphism was estimated using crude ORs, with the corresponding 95%CIs. In addition, Z-test was also used, and the P value<0.05 indicated statistical significance for the association. The pooled ORs were performed for co-dominant model (BB *vs* bb, Bb *vs* bb), dominant model (BB+Bb *vs* bb), and recessive model (BB *vs* Bb+bb).

Both the Cochran's Q statistic (Cochran, 1954) to test for heterogeneity and the I^2 statistic to quantify the proportion of the total variation due to heterogeneity (Higgins et al., 2003) were calculated. A P value of more than the nominal level of 0.10 for the Q statistic or the I-squared less than 50% indicated a lack of heterogeneity across studies, allowing for the use of a fixed-effects model (the Mantel-Haenszel method) (MANTEL and HAENSZEL,1959); otherwise, the random-effects model (the DerSimonian and Laird method) was used (DerSimonian and Laird, 1986). The Galbraith plot was used to detect the potential sources of heterogeneity (Galbraith,1988). Heterogeneity was also explored using subgroup analysis with ethnicity (Asian/Caucasian), source of controls (PCC/HCC), study sample size (>1000 subjects/≤1000 subjects) and HWE in controls (Yes/No).

Sensitivity analyses were performed to assess the stability of the results, namely, a single case-control study in this meta-analysis was omitted each time to reflect the influence of the individual data set to the pooled OR. Several methods were used to assess the potential publication bias. Visual inspection of funnel plot asymmetry was conducted. The Begg's rank correlation method (Begg and Mazumdar,1994) and the Egger's

weighted regression method (Egger et al., 1997) were used to statistically assess publication bias ($P < 0.05$ was considered statistically significant). All analyses were done using STATA software, version 11.0 (STATA Corp., College Station, TX, USA). All the P values were two-sided.

Results

Characteristics of studies

We identified 72 relevant studies when searched the databases. Through screening the title and reading the abstract and the entire article, a total of fourteen eligible studies (Slatter et al., 2001; Speer et al., 2001; Slattery et al., 2004; Park et al., 2006; Flugge et al., 2007; Kadiyska et al., 2007; Slattery et al., 2007; Parisi et al., 2008; Theodoratou et al., 2008; Jenab et al., 2009; Li et

al., 2009; Hughes et al., 2011; Mahmoudi et al., 2011; Gunduz et al., 2012) involving 10822 cases and 11779 controls were included in the pooled analyses finally. The characteristics of selected studies are summarized in Table 1. There were eleven studies of Caucasian patients, three studies of Asian patients. Studies had been carried out in USA, Hungary, South Korea, Bulgaria, Germany, Spain, UK, China, Czech, Iran and Turkey. The cases definition used in the individual studies were pathologically or histologically diagnosed with CRC. Controls were mainly healthy populations and matched for age and/or sex, of which five were population-based and nine were hospital-based. There was a wide variation in the VDR BsmI B allele frequency among different ethnicities, ranging from 5.0% in an Asian population (Park et al., 2006) to 74.0% in another Asian population (Li et al., 2009). The distributions of genotypes in the controls of all studies

Table 1. Characteristics of Studies Included in this Meta-Analysis

First author Reference	Year	Country	Ethnicity	Source of Controls	Sample size (case/control)	Genotyping Methods	MAF in Controls	HWE
Slatter	2001	USA	Caucasian	PCC	250/364	PCR-RFLP	0.43	Yes
Speer	2001	Hungary	Caucasian	HCC	56/112	PCR-RFLP	0.43	Yes
Slattery	2004	USA	Caucasian	PCC	1959/2174	PCR-RFLP	0.4	No
Park	2006	South Korea	Asian	HCC	190/320	PCR-RFLP	0.05	Yes
Kadiyska	2007	Bulgaria	Caucasian	HCC	133/94	PCR-RFLP	0.46	Yes
Flügge	2007	Germany	Caucasian	HCC	256/256	PCR-RFLP	0.36	Yes
Slattery	2007	USA	Caucasian	PCC	2313/2902	Taqman	0.4	Yes
Parisi	2008	Spain	Caucasian	HCC	170/120	PCR-RFLP	0.37	Yes
Theodoratou	2008	UK	Caucasian	PCC	2984/3038	Microarray	0.41	No
Li	2009	China	Asian	HCC	200/200	PCR-RFLP	0.74	No
Jenab	2009	Mixed	Caucasian	PCC	1091/1091	Taqman	0.43	Yes
Hughes	2011	Czech	Caucasian	HCC	725/614	AS-PCR	0.39	Yes
Mahmoudi	2011	Iran	Asian	HCC	452/452	PCR-RFLP	0.42	Yes
Gunduz	2012	Turkey	Caucasian	HCC	43/42	PCR-RFLP	0.5	No

*Abbreviations: HCC, hospital-based case-control; PCC, population-based case-control; PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism; AS-PCR, Allele-specific PCR; MAF, minor allele frequency; HWE, Hardy–Weinberg equilibrium
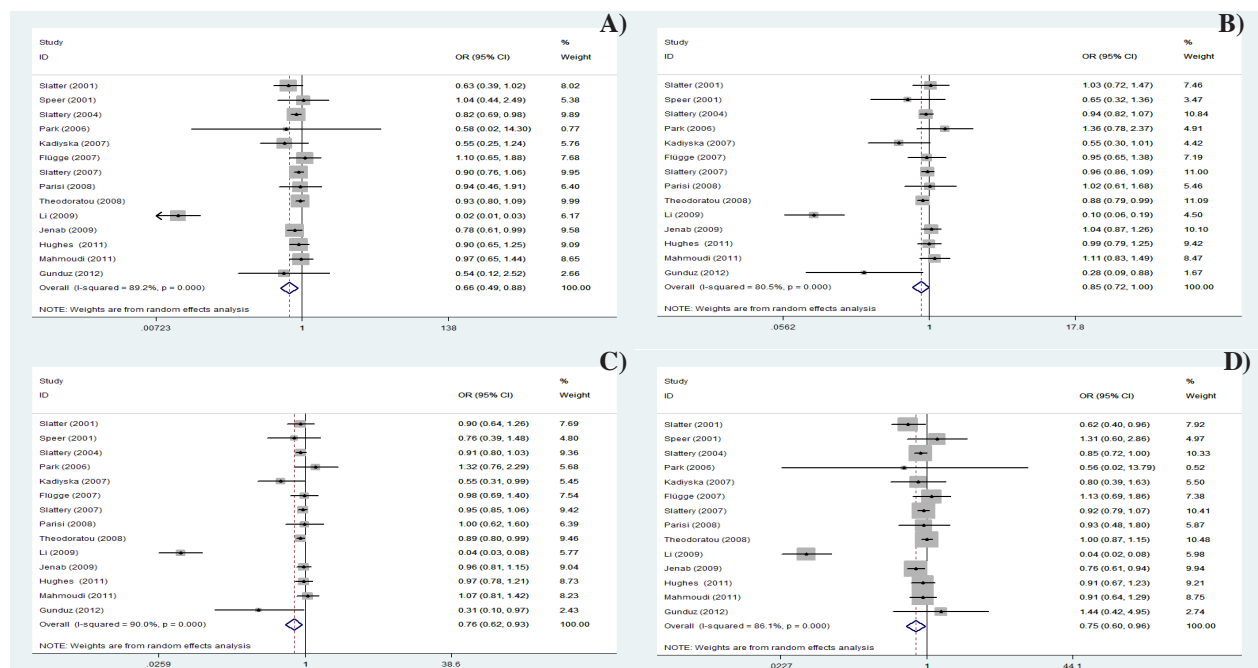

Figure 1. Forest Plots of Colorectal Cancer Risk Associated with the VDR BsmI Polymorphism. The squares and horizontal lines correspond to the study- specific OR and 95% CI. The area of the squares reflects the study-specific weight (inverse of the variance). The diamonds represent the pooled OR and 95% CI. **A)** BB *vs* bb; **B)** Bb *vs* bb; **C)** BB+Bb *vs* bb; **D)** BB *vs* Bb+bb

Table 2. Quantitative Analyses of the VDR Bsm I Polymorphism on the Colorectal Cancer (CRC) Risk

Genetic model Variables	Sample size		Homozygote BB *vs* bb		Heterozygote Bb *vs* bb		Dominant model BB+Bb *vs* bb		Recessive model BB *vs* Bb+bb	
	N[a]	Case/control	OR(95%CI)	*p* value[b]	OR(95%CI)	*p* value[b]	OR(95%CI)	*p* value[b]	OR(95%CI)	*p* value[b]
Total	14	10822/11779	0.66(0.49, 0.88)	0	0.85(0.72, 1.00)	0	0.76(0.62, 0.93)	0	0.75(0.60, 0.96)	0
Ethnicity										
Asians	3	842/972	0.19(0.01, 5.58)	0	0.54(0.13, 2.24)	0	0.40(0.06, 2.84)	0	0.26(0.02, 3.31)	0
Caucasians	11	9980/10807	0.87(0.80, 0.94)	0.77	0.93(0.88, 0.99)	0.332	0.92(0.87, 0.97)	0.619	0.90(0.84, 0.97)	0.39
Source of controls										
HCCc	9	2225/2210	0.51(0.22, 1.18)	0	0.67(0.44, 1.03)	0	0.60(0.34, 1.06)	0	0.68(0.36, 1.31)	0
PCCc	5	8597/9569	0.86(0.79, 0.94)	0.421	0.94(0.88, 1.00)	0.576	0.92(0.87, 0.98)	0.928	0.89(0.82, 0.97)	0.086
Study sample size										
>1000	5	9072/9819	0.87(0.80, 0.95)	0.699	0.94(0.88, 1.00)	0.586	0.92(0.87, 0.98)	0.9	0.90(0.84, 0.98)	0.256
≤1000	9	1750/1960	0.49(0.20, 1.18)	0	0.67(0.42, 1.05)	0	0.59(0.33, 1.09)	0	0.65(0.33, 1.29)	0
HWEd in controls										
Yes	10	5636/6325	0.86(0.77, 0.96)	0.81	0.99(0.91, 1.07)	0.611	0.96(0.89, 0.99)	0.733	0.87(0.79, 0.96)	0.652
No	4	5186/5454	0.29(0.12, 0.74)	0	0.48(0.30, 0.79)	0	0.36(0.19, 0.69)	0	0.47(0.23, 0.98)	0

*[a]Number of comparisons; [b]P value of Q-test for heterogeneity test. Random-effects model was used when P value for heterogeneity test <0.1; otherwise, fixed-effects model was used; [c]HCC, hospital-based case-control; PCC, population-based case-control; [d]HWE, Hardy–Weinberg equilibrium
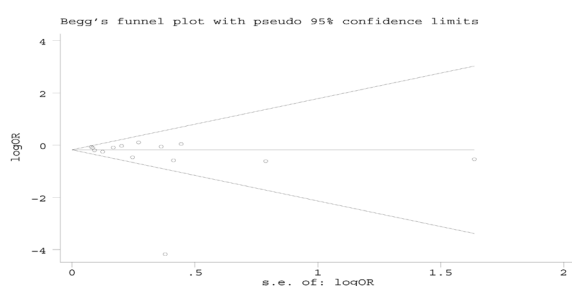

Figure 2. Galbraith Plot of the Association between VDR BsmI Polymorphism and Colorectal Cancer Risk (The Study outside the range between -2 and 2 was seen as the outlier and the Major source of Heterogeneity)

Figure 3. Funnel Plot for Publication Bias Test. (BB vs. bb). Each point represents a separate study for the indicated association

were in agreement with HWE except for four studies (Slattery et al., 2004; Theodoratou et al., 2008; Li et al., 2009; Gunduz et al., 2012).

Quantitative synthesis

Table 2 listed the main results of this meta-analysis and Figure 1A-1D showed the association of CRC risk with VDR BsmI polymorphism. Overall, the variant genotype (BB) of the BsmI was associated with a lower CRC risk when compared with the wild-type bb homozygote (OR=0.66, 95%CI=0.49-0.88). Similarly, a decreased CRC risk was also found in the dominant and recessive models (dominant model, OR=0.76, 95%CI=0.62-0.93; recessive model, OR=0.75, 95%CI=0.60-0.96).

On the basis of the potential underestimation of the true effect of the polymorphism on the CRC risk, we stratified these studies according to ethnicity, source of controls and study sample size. Different ethnicities were categorized as Asian and Caucasian. Different source of controls were defined as HCC and PCC. When stratifying for ethnicity, we found that the BsmI polymorphism was associated with a lower CRC risk among Caucasians (BB *vs* bb, OR=0.87, 95%CI=0.80-0.94; Bb *vs* bb, OR=0.93, 95%CI=0.88-0.99; dominant model, OR=0.92, 95%CI=0.87-0.97; recessive model, OR=0.90, 95%CI=0.84-0.97). In the subgroup analysis by source of controls, the significant association was observed among population-based studies (BB *vs* bb, OR=0.86, 95%CI=0.79-0.94; dominant model, OR=0.92, 95%CI=0.87-0.98; recessive model,
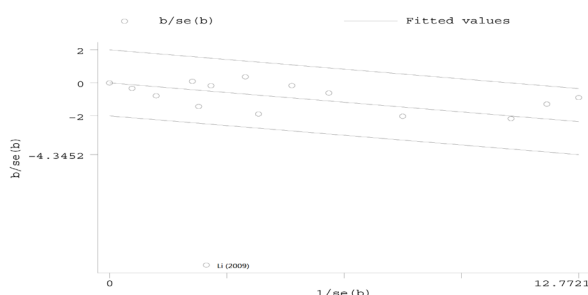
OR=0.89, 95%CI=0.82-0.97). When stratifying for study sample size, we found that the BsmI polymorphism was associated with a decreased CRC risk among studies with large study sample size (>1000 subjects) (BB *vs* bb, OR=0.87, 95%CI=0.80-0.95; dominant model, OR=0.92, 95%CI=0.87-0.98; recessive model, OR=0.90, 95%CI=0.84-0.98).

Heterogeneity and sensitivity analyses

Significant heterogeneity between studies was observed in overall comparisons and also subgroup analyses. To identify which of the 14 studies may be sources of heterogeneity, we used a Galbraith plot to assess the potential sources of heterogeneity. Li et al's study (Li et al., 2009) was found to be contributor of heterogeneity for the BsmI polymorphism (Figure 2). We re-evaluated the association after excluding the outlier study with reduced heterogeneity under various genetic models (BB *vs* bb: Pheterogeneity =0.87; Bb *vs* bb: Pheterogeneity=0.29; dominant model: Pheterogeneity=0.54; recessive model: Pheterogeneity=0.56). Moreover, in the overall analysis, the significant association between the polymorphism and CRC risk was also detected, which was similar with the result when the outlier study was included (data not shown). In the sensitivity analysis, the influence of each study on the pooled OR was examined by repeating the meta-analysis while omitting each study, one at a time. This procedure confirmed the stability of our overall results. In addition, when excluding the studies that were not in HWE, the results were persistent and robust (Table 2).

Publication bias

Funnel plot, Begg's and Egger's tests were performed to evaluate publication bias of the literature on CRC. Figure 3 displayed a funnel plot that examined the VDR BsmI polymorphism and overall CRC risk included in the meta-analysis in the homozygous comparison. The shape of funnel plots did not reveal any evidence of funnel plot asymmetry. The statistical results still did not show publication bias (BB *vs* bb: Begg's test *P*=0.16, Egger's test *p*=0.21; Bb *vs* bb: Begg's test *p*=0.16, Egger's test *p*=0.20; dominant model: Begg's test *p*=0.15, Egger's test *p*=0.21; recessive model: Begg's test *p*=0.58, Egger's test *p*=0.31).

Discussion

The present meta-analysis, including 10822 cases and 11779 controls from 14 case - control studies, explored the association between the BsmI polymorphism of the VDR gene and CRC risk. Therefore a larger sample size and increased statistical power could be obtained. Overall, we found that the BsmI polymorphism was associated with a decreased CRC risk. When stratifying for ethnicity, source of controls, and study sample size, the significant association was observed among Caucasians, population-based studies and studies with large study sample size (>1000 subjects). Limiting the analysis to the studies within Hardy-Weinberg equilibrium, the results were persistent and robust.

Biological and epidemiological data suggest that vitamin D levels may influence cancer development. Vitamin D, a steroid hormone, exerts its biological effects through the active metabolite 1a , 25 dihydroxy-vitamin D3 (1,25 (OH) 2 D 3), whose activity requires binding to the VDR. The VDR is an intracellular hormone receptor that specifically binds the biologically active form of vitamin D, and interacts with specific nucleotide sequences (response elements) of target genes to produce a variety of biologic effects (Raimondi et al., 2009). The VDR gene is located on chromosome 12q12-q14 and several SNPs have been identified that may influence cancer risk. The most frequently studied SNPs are the restriction fragment length polymorphisms FokI and BsmI , as defined by the endonucleases FokI and BsmI, respectively. For example, Rasool et al (Rasool et al., 2013) recently studied the relationship between the VDR Fok I polymorphism and CRC risk in Kashmir and revealed that a possible role of Fok I polymorphism in the etiology of CRC in Kashmir. Since Slatter et al (Slatter et al., 2001) first examined the association between the VDR polymorphism and the risk of CRC, a number of studies have been conducted to evaluate the role of BsmI polymorphism in the VDR gene on CRC risk. However, the results remain conflicting rather than conclusive. In recent years, two meta-analyses (Touvier et al., 2011; Bai et al., 2012) were published to assess the association and revealed that this polymorphism was associated with a decreased CRC risk. However, the two studies had some limitations, such as relatively small sample size. In Bai et al's meta-analysis (Bai et al., 2012), a total of 12 original studies were included for the VDR BsmI polymorphism and CRC risk. However, upon

careful study, we found that several studies which detected VDR BsmI polymorphism and colorectal adenoma risk were also included in the 12 original studies. Moreover, a number of studies were published after that period. Therefore, an updated meta-analysis of association between VDR BsmI polymorphism and CRC risk was of great value.

Our results were consistent with that of the two previous meta-analyses (Touvier et al., 2011; Bai et al., 2012), which further confirmed the conclusions of the two previous meta-analyses. However, our results were not consistent with several meta-analysis about VDR BsmI polymorphism and other cancer risk. For instance, Mahmoudi et al. (2014) recently found that Vitamin D Metabolism -Related Gene (including VDR) Variants was not associated with Risk of Colorectal Cancer in Iranian population. In recent, Zhang et al. (2013) performed a meta-analysis to detect VDR BsmI polymorphism and ovarian cancer risk and found that there was no association between VDR BsmI polymorphism and susceptibility to ovarian cancer in Caucasians. Moreover, Du et al. (2014) performed an updated meta-analysis involving 23,020 subjects to re-evaluate vitamin D receptor gene BsmI polymorphism and breast cancer risk. Similarly, this meta-analysis suggested that there were no associations between VDR BsmI polymorphism and breast cancer. Although the reasons for this difference are as yet unknown, some possibilities should be considered. First, those gene-variant associations vary in different kinds of diseases and may result from the different mechanisms of carcinogenesis among different kinds of tumor. Second, different ethnic composition may contribute to the discrepancy. Different meta-analyses included different original studies which were performed in different races and the ethnic composition in different meta-analyses may be diversity. Third, some methodological diversity, such as inclusion criteria, the quality of original studies, selection bias Type I error and study sample size, also can contribute to the discrepancy.

Because the allele frequencies of polymorphisms and their effects on the cancer risk were diverse in the different ethnicities, we carried out subgroup analysis by ethnicity. The results demonstrated that VDR BsmI polymorphism was associated with a decreased CRC risk among Caucasians, while there was no association between VDR BsmI polymorphism and CRC risk among Asians. The null result may be due to the limited number of studies with only three studies from Asian available in this meta-analysis. Moreover, results of meta-analyses often depend on control selection procedures (Benhamou et al., 2002). Different controls source may be a confounding factor which may impact on the conclusion of our study because of case-control studies. In order to eliminate interference from the confounding factor, we performed subgroup analysis by source of controls. Our results showed that the significant association between VDR BsmI polymorphism and CRC was observed among PCC, but not among HCC. This may be due to that the HCC studies have some selection biases because such controls might be ill-related population, and may not be a representative of the general population.

One of the major concerns in a sound meta-analysis is publication bias due to selective publication of reports. In the current study, Begg's funnel plot and Egger's test were performed to evaluate this problem. Both the shape of funnel plots and statistical results did not show publication bias. Another important issue for any meta-analysis is the degree of heterogeneity that exists between the component studies because non-homogeneous data are liable to results in misleading results. In the present study, the Q-test and I 2 statistics were carried out to test the significance of heterogeneity. Obvious heterogeneity between studies was observed in overall comparisons and also some subgroup analyses. In an attempt to find the sources of heterogeneity, a Galbraith plot was drawn, and one study was thought to serve as the main contributors. The heterogeneity was significantly reduced when excluding the outlier study. Moreover, we re-analyzed the association after excluding the outlier study; the conclusion was still consistent in overall comparisons.

Some limitations of this meta-analysis should be addressed. First, the number of studies and the number of subjects in the studies included in the meta-analysis by specific subgroups were small (such as Asian studies), thus, caution should be adopted when explaining our results. Second, our meta-analysis was based on unadjusted OR estimates because not all published studies presented adjusted ORs or when they did, the ORs were not adjusted by the same potential confounders, such as age, sex, ethnicity and exposures. Lacking of the information for the data analysis may cause serious confounding bias. Third, there was significant between-study heterogeneity from studies of the polymorphism, and the genotype distribution also showed deviation from HWE in some studies.

In conclusion, this meta-analysis reveals that the VDR BsmI polymorphism may have a potential protective effect on CRC. Since limited studies were from Asian population, it is critical that larger and well-designed multicentric studies, especially Asian studies, should be performed to re-evaluate the associations. Moreover, further studies estimating the effect of gene-environment interactions may eventually provide a better, comprehensive understanding of the associations between the VDR BsmI polymorphism and CRC risk.

References

Bai YH, Lu H, Hong D, et al (2012). Vitamin D receptor gene polymorphisms and colorectal cancer risk: a systematic meta-analysis. *World J Gastroenterol*, **18**, 1672-9.

Beelman CA, Parker R (1995). Degradation of mRNA in eukaryotes. *Cell*, **81**, 179-83.

Begg CB, Mazumdar M (1994). Operating characteristics of a rank correlation test for publication bias. *Biometrics*, **50**, 1088-101.

Benhamou S, Lee WJ, Alexandrie AK, et al (2002). Meta- and pooled analyses of the effects of glutathione S-transferase M1 polymorphisms and smoking on lung cancer risk. *Carcinogenesis*, **23**, 1343-50.

Cochran WG (1954). The combination of estimates from different experiments. Biometrics DerSimonian R and Laird N (1986). Meta-analysis in clinical trials. *Control Clin Trials*, **7**, 177-88.

Du Y, Hu L, Kong F, et al (2014). Lack of association between vitamin D receptor gene BsmI polymorphism and breast cancer risk: an updated meta-analysis involving 23,020 subjects. *Tumour Biol*, **35**, 2087-93.

Egger M, Davey SG, Schneider M, et al (1997). Bias in meta-analysis detected by a simple, graphical test. *BMJ*, **315**, 629-34.

Flugge J, Krusekopf S, Goldammer M, et al (2007). Vitamin D receptor haplotypes protect against development of colorectal cancer. *Eur J Clin Pharmacol*, **63**, 997-1005.

Galbraith RF (1988). A note on graphical presentation of estimated odds ratios from several clinical trials. *Stat Med*, **7**, 889-94.

Garland CF, Garland FC (1980). Do sunlight and vitamin D reduce the likelihood of colon cancer. *Int J Epidemiol*, **9**, 227-31.

Garland CF, Gorham ED, Mohr SB, et al (2009). Vitamin D for cancer prevention: global perspective. *Ann Epidemiol*, **19**, 468-83.

Giovannucci E (2007). Epidemiological evidence for vitamin D and colorectal cancer. *J Bone Miner Res*, **22**, 81-5.

Gunduz M, Cacina C, Toptas B, et al (2012). Association of vitamin D receptor gene polymorphisms with colon cancer. *Genet Test Mol Biomarkers*, **16**, 1058-61.

Higgins JP, Thompson SG, Deeks JJ, et al (2003). Measuring inconsistency in meta-analyses. *BMJ*, **327**, 557-60.

Hughes DJ, Hlavata I, Soucek P, et al (2011). Variation in the vitamin D receptor gene is not associated with risk of colorectal cancer in the Czech Republic. *J Gastrointest Cancer*, **42**, 149-54.

Jemal A, Bray F, Center MM, et al (2011). Global cancer statistics. *CA Cancer J Clin*, **61**, 69-90.

Jenab M, McKay J, Bueno-de-Mesquita HB, et al (2009). Vitamin D receptor and calcium sensing receptor polymorphisms and the risk of colorectal cancer in European populations. Cancer Epidemiol. *Biomarkers Prev*, **18**, 2485-91.

Jones G, Strugnell SA, DeLuca HF (1998). Current understanding of the molecular actions of vitamin D. *Physiol Rev*, **78**, 1193-231.

Kadiyska T, Yakulov T, Kaneva R, et al (2007). Vitamin D and estrogen receptor gene polymorphisms and the risk of colorectal cancer in Bulgaria. *Int J Colorectal Dis*, **22**, 395-400.

Kim HS, Newcomb PA, Ulrich CM, et al (2001). Vitamin D receptor polymorphism and the risk of colorectal adenomas: evidence of interaction with dietary vitamin D and calcium. Cancer Epidemiol. *Biomarkers Prev*, **10**, 869-74.

Li C, Li Y, Gao LB, et al (2009). Vitamin D receptor gene polymorphisms and the risk of colorectal cancer in a Chinese population. *Dig Dis Sci*, **54**, 634-9.

Mahmoudi T, Karimi K, Arkani M, et al (2014). Lack of associations between vitamin D metabolism-related gene variants and risk of colorectal cancer. *Asian Pac J Cancer Prev*, **15**, 957-61.

Mahmoudi T, Karimi K, Mohebbi SR, et al (2011). Start codon FokI and intron 8 BsmI variants in the vitamin D receptor gene and susceptibility to colorectal cancer. *Mol Biol Rep*, **38**, 4765-70.

Mantel N, Haenszel W (1959). Statistical aspects of the analysis of data from retrospective studies of disease. *J Natl Cancer Inst*, **22**, 719-48.

Parisi E, Rene JM, Cardus A, et al (2008). Vitamin D receptor levels in colorectal cancer. Possible role of BsmI polymorphism. *J Steroid Biochem Mol Biol*, **111**, 87-90.

Park K, Woo M, Nam J, et al (2006). Start codon polymorphisms in the vitamin D receptor and colorectal cancer risk. *Cancer Lett*, **237**, 199-206.

Pilz S, Tomaschitz A, Obermayer-Pietsch B, et al (2009). Epidemiology of vitamin D insufficiency and cancer mortality. *Anticancer Res*, **29**, 3699-704.

Raimondi S, Johansson H, Maisonneuve P, et al (2009). Review and meta-analysis on vitamin D receptor polymorphisms and cancer risk. *Carcinogenesis*, **30**, 1170-80.

Rasool S, Kadla SA, Khan T, et al (2013). Association of a VDR gene polymorphism with risk of colorectal cancer in Kashmir. *Asian Pac J Cancer Prev*, **14**, 5833-7.

Slatter ML, Yakumo K, Hoffman M, et al (2001). Variants of the VDR gene and risk of colon cancer (United States). *Cancer Causes Control*, **12**, 359-64.

Slattery ML, Murtaugh M, Caan B, et al (2004). Associations between BMI, energy intake, energy expenditure, VDR genotype and colon and rectal cancers (United States). *Cancer Causes Control*, **15**, 863-72.

Slattery ML, Wolff RK, Herrick JS, et al (2007). IL6 genotypes and colon and rectal cancer. *Cancer Causes Control*, **18**, 1095-105.

Speer G, Cseh K, Winkler G, et al (2001). Oestrogen and vitamin D receptor (VDR) genotypes and the expression of ErbB-2 and EGF receptor in human rectal cancers. *Eur J Cancer*, **37**, 1463-8.

Sung JJ, Lau JY, Goh KL, et al (2005). Increasing incidence of colorectal cancer in Asia: implications for screening. *Lancet Oncol*, **6**, 871-6.

Theodoratou E, Farrington SM, Tenesa A, et al (2008). Modification of the inverse association between dietary vitamin D intake and colorectal cancer risk by a FokI variant supports a chemoprotective action of Vitamin D intake mediated through VDR binding. *Int J Cancer*, **123**, 2170-9.

Thorne J, Campbell MJ (2008). The vitamin D receptor in cancer. *Proc Nutr Soc*, **67**, 115-27.

Touvier M, Chan DS, Lau R, et al (2011). Meta-analyses of vitamin D intake, 25-hydroxyvitamin D status, vitamin D receptor polymorphisms, and colorectal cancer risk. *Cancer Epidemiol Biomarkers Prev*, **20**, 1003-16.

Uitterlinden AG, Fang Y, van Meurs JB, et al (2004). Vitamin D receptor gene polymorphisms in relation to Vitamin D related disease states. *J Steroid Biochem Mol Biol*, **89-90**, 187-93.

van der Rhee H, Coebergh JW, de Vries E (2009). Sunlight, vitamin D and the prevention of cancer: a systematic review of epidemiological studies. *Eur J Cancer Prev*, **18**, 458-75.

Zhang Y, Tong SC, Guan LH, et al (2013). Meta-analysis of the relation between vitamin D receptor gene BsmI polymorphism and susceptibility to ovarian cancer. *Tumour Biol*, **34**, 3317-21.

Zhao P, Dai M, Chen W, et al (2010). Cancer trends in China. *Jpn J Clin Oncol*, **40**, 281-5.