Medicine®

SYSTEMATIC REVIEW AND META-ANALYSIS

OPEN

Association of Vitamin D Receptor Cdx-2 Polymorphism With Cancer Risk

A Meta-Analysis

Zhi-Ming Dai, MD, Yu-Lang Fei, PhD, Wang-Gang Zhang, MD, PhD, Jie Liu, MD, Xing-Mei Cao, MD, Qiu-Min Qu, PhD, Yan-Chun Li, MD, Shuai Lin, MD, Meng Wang, MD, and Zhi-Jun Dai, MD, PhD

Abstract: Vitamin D receptor (VDR) Cdx-2 polymorphism (rs11568820) has been indicated to be associated to cancer susceptibility. However, published studies reported mixed results. This meta-analysis was conducted to get a more accurate estimation of the association between Cdx-2 polymorphism and cancer risk.

We identified 25 independent studies with a total of 34,018 subjects published prior to March 2015. Summary odds ratios (ORs) and 95% confidence intervals (CIs) were used to evaluate the susceptibility to cancer. Separate analyses were conducted on features of the population such as ethnicity, source of controls, and cancer types.

Meta-analysis results showed that Cdx-2 polymorphism significantly increased cancer risk in the homozygous model in overall analysis. According to the further stratified analysis, significant association was found between Cdx-2 variant and cancer risk in American-Africans in the homozygous, recessive, and dominant comparison models. However, no significant associations were found in Caucasians and Asians. When stratified by different cancer types, significant association was observed between Cdx-2 variant and an increased risk of colorectal cancer in the homozygous, recessive, and dominant models. In addition, ovarian cancer susceptibility increased based on the homozygous and dominant comparison models.

Our study indicated that VDR Cdx-2 polymorphism was associated with an increased cancer risk, particularly in American-Africans, colorectal, and ovarian cancers. However, other factors may impact on the association. Further multicenter studies are needed to confirm the effects of Cdx-2 polymorphism on cancer susceptibility.

(*Medicine* 94(33):e1370)

Abbreviations: CI = confidence interval, CNKI = Chinese National Knowledge Infrastructure, HWE = Hardy–Weinberg equilibrium, NOS = the Newcastle–Ottawa Scale, OR = odds ratio, SNP = single-nucleotide polymorphism, VDR = vitamin D receptor.

INTRODUCTION

Vitamin D is an essential fat-soluble vitamin which not only maintains the stability of the extracellular calcium and phosphorous concentration by regulating bone turnover and absorption in the gut but also regulates the growth, differentiation, and apoptosis of many normal or tumor tissue cells.[1] Vitamin D receptor (VDR) binds to 1,25-hydroxy vitamin D, the active form of vitamin D, and mediates its biological activity. VDR is active in over 30 different human tissues as an intracellular nuclear receptor and regulates gene expression as a ligand-activated transcription factor.[2] Single nucleotide polymorphisms exist in human genome widely. The polymorphisms of the VDR gene have been reported to exert functional effects on the VDR expression at the transcriptional level and on the receptor affinity.[3] The Cdx-2 (rs11568820) polymorphism of VDR is located in the promoter region of the VDR gene, which carries a G to A sequence change and affects the function of the transcription factor CDX.[4]

Cancer is currently one of the global public health problems, which threatens to human health seriously. Biological and epidemiological studies suggest that carcinogenesis is a multivariate and complicated process because of the interactions between genetic and environmental factors.[5] Previous studies suggested that lower mean serum vitamin D levels or vitamin D deficiency is common in oncology patients and correlates with advanced stage disease.[6,7] Other studies indicated that VDR plays crucial roles in cancers such as regulation of the immune function and modulation of cell proliferation and differentiation.[8,9] Associations of VDR gene variants with different types of cancer have been widely researched. Kupfer et al[10] found a significant association between the VDR polymorphisms and vitamin D intake in colorectal cancer. Ditsch et al[11] proved that the potential anticancer function of vitamin D might be mediated by VDR expression and that VDR can influence cancer predisposition through binding to vitamin D.

Cdx-2 is one of the common polymorphisms within the VDR gene promoter region that may impact on VDR expression and vitamin D intake. It has been hypothesized that the Cdx-2 polymorphism exerts function in carcinogenesis and the association has been investigated between the Cdx-2 variants and several different types of cancer by numerous studies in the past years. However, previous studies results are inconsistent.[12–33] In addition, an earlier meta-analysis found that the Cdx-2 AA

Editor: Jimmy Efird.
Received: April 21, 2015; revised: June 30, 2015; accepted: July 21, 2015.
From the Department of Hematology (Z-MD, W-GZ, JL, X-MC); Department of Anesthesia (Z-MD), and Department of Oncology, Second Affiliated Hospital of Xi'an Jiaotong University, Xi'an, China (SL, MW, Z-JD); Department of Neurology, First Affiliated Hospital of Xi'an Jiaotong University, Xi'an, China (Y-LF, Q-MQ); and Department of Hematological Genetics, Shaanxi Provincial People's Hospital, Xi'an, China (Y-CL).
Correspondence: Zhi-Jun Dai, Department of Oncology, Second Affiliated Hospital of Xi'an Jiaotong University, Xi'an 710004, China (e-mail: dzj0911@126.com).
Z-MD, Y-LF, and W-GZ have contributed equally to this work and share joint first authorship.
This study was supported by the National Natural Science Foundation of China (No. 81471670); the Fundamental Research Funds for the Central Universities, China (No. 2014qngz-04), and the Science and Technology Foundation of Shaanxi Province, China (No. 2014K11020107).
The authors have no conflicts of interest to disclose.

ISSN: 0025-7974
DOI: 10.1097/MD.0000000000001370

homozygote carriers significant elevated cancer risk.[34] But this study only included articles that were performed in Caucasians and African Americans, and was lack of data on Asian populations. In recent years, there were a lot of new literatures published. Therefore, we conduct this meta-analysis on all eligible case–control studies to comprehensively estimate the relationship between the Cdx-2 polymorphism and cancer risk.

MATERIALS AND METHODS

Publication Search and Inclusion Criteria

We searched the Web of knowledge, PubMed, and Chinese National Knowledge Infrastructure databases for studies published prior to March 2015 (last search: March 31, 2015). The keywords searching were conducted with and without MeSH terms for ''vitamin D receptor/VDR,'' ''polymorphism,'' and ''cancer.'' The languages were limited to English and the subjects were human. Studies included in our meta-analysis must meet the criteria as follows: (1) evaluation of the association of VDR Cdx-2 polymorphism and cancer risk; (2) case–control design; (3) available information on genotype frequency. The following were the exclusion criteria: (1) repeat studies, review, and abstracts; (2) study design was based on the family; (3) the genotype distribution of the control population was not consistent with Hardy–Weinberg equilibrium (HWE).

Data Extraction

Initially, 2 investigators (Z-MD and Y-LF) independently checked all potentially relevant studies and disagreements were resolved through discussion with a third researcher (Z-JD). We extracted the following items for each article: first author, years of publication, original country, subjects' ethnicity, cancer types, source of control, genotyping method, total number of cases and controls, and number of different genotypes in cases and controls. All data came from published articles. All cancers were confirmed by histology or pathology. The noncancer controls had no present evidence of any malignant disease. Controls were identified through random selections and the source of controls was either population based or hospital based. Controls and cancer cases used the same gene detection method and was matched in age and sex and so on. Therefore, all the case and control groups were well controlled. The Newcastle–Ottawa Scale (NOS) was used to assess the quality of included studies.[35]

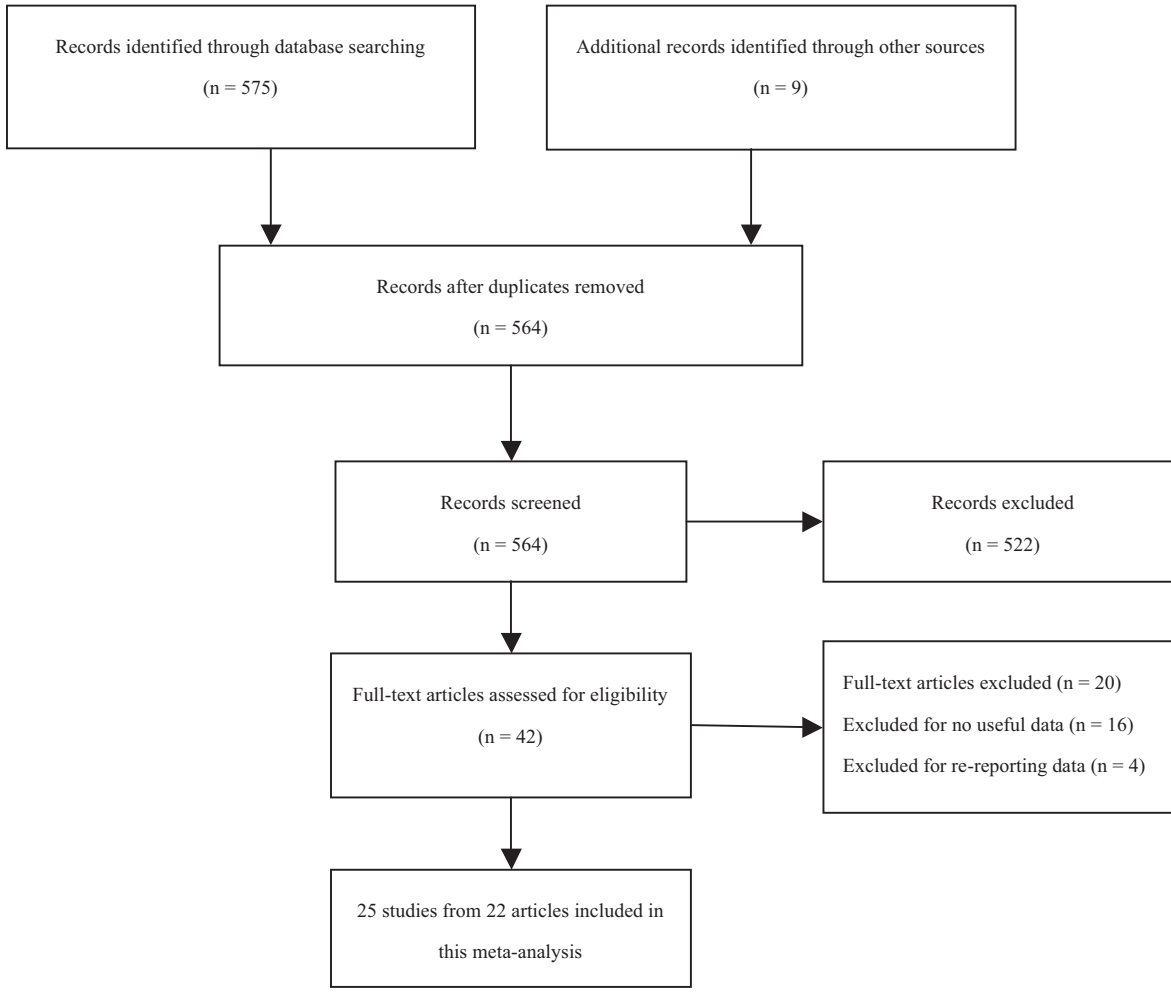

FIGURE 1. Preferred reporting items for systematic reviews and meta-analyses flow diagram of the literature review process for Cdx-2 polymorphism and cancer.

Statistical Analysis

We used odds ratios (OR) and 95% confidence intervals (CIs) to evaluate cancer risk associated with the VDR Cdx-2 polymorphism. Statistical heterogeneity between studies was evaluated with the I^2 test, higher I^2 values means higher levels of heterogeneity ($I^2 = 75\%$ to 100%: extreme heterogeneity; $I^2 = 50\%$ to 75%: large heterogeneity; $I^2 = 25\%$ to 50%: moderate heterogeneity; $I^2 < 25\%$: no heterogeneity). In heterogeneity evaluation, when the P value ≥ 0.10, the fixed-effects model would be used; if $P < 0.10$, a random-effects model was used. To estimate the cancer site-specific and ethnicity-specific effects, subgroup analyses were performed by cancer types, source of controls, and ethnicity. Sensitivity analysis was performed to assess the stability of the final results. In order to assess the influence of each study to the pooled OR, risk assessment was tested by sequentially omitting 1 individual study at a time. Sensitivity analysis determines whether the individual data in fact have a major effect on the results of the review. The Egger's test and Begg's test were adopted to assess publication bias. The meta-analysis assessed the following genetic models: dominant model (AA + GA vs GG), recessive model (AA vs GG + GA), homozygote comparison (AA vs GG), heterozygote comparison (GA vs GG), and allele comparison (A vs G). All analysis was performed by the Stata version 12.0. All the P values were 2-sided.

RESULTS

Study Characteristics

There were 584 articles obtained by keyword search and manual search. The flow chart for studies selection process was shown in Figure 1. Overall, our study included 27 independent studies that contained detailed genotype distribution data, among which 2 different studies were excluded from our meta-analysis because the genotype distributions of the control group departed from HWE.[16,21] Finally, a total of 25 studies from 22 published articles, involving 16,269 cases and 17,749 cancer-free controls were included in this meta-analysis. Eligible studies presented data for several different cancer types including colorectal, prostate, skin, breast, ovary, brain, and esophageal cancer. Among these studies, 15 studies were based on Caucasian, 3 on Asian, and 2 on African-American and 5 on mixed ethnicities. The NOS score of all articles are not <6, so that each included literature was a high-quality study. The characteristics of the eligible studies are presented in Table 1. The genotype distributions are shown in Table 2,

TABLE 1. Characteristics of the Studies Included in the Meta-Analysis

First Author	Year	Country	Cancer Type*	Ethnicity	Genotyping Method	Source of Control	Case/Control	*P* of HWE	NOS
Woodford-Richens	2001	UK	CRC	Caucasian	SSP-PCR	PB	49/51	0.39	6
John	2005	USA	PC	Caucasian	Taqman	PB	417/435	0.75	9
Han	2006	USA	SC	Caucasian	Taqman	PB	781/853	0.68	8
Mikhak	2007	USA	PC	Mix	Taqman	PB	688/689	0.15	8
Lurie	2007a	USA	OC	Caucasian	Taqman	PB	70/145	0.75	8
	2007b	USA	OC	Asian	Taqman	PB	92/171	**0.03**	8
Flügge	2007	Germany	CRC	Caucasian	PCR-RFLP	PB	256/256	0.08	9
Torkko	2008a	USA	PC	Caucasian	Taqman	PB	444/488	0.99	7
	2008b	USA	PC	Hispanic	Taqman	PB	141/273	**0.05**	7
Ochs-Balcom	2008	USA	CRC	Mix	Taqman	PB	250/246	0.95	8
Theodoratou	2008	UK	CRC	Caucasian	MassArray	PB	1996/2037	0.54	8
Abbas	2008	Germany	BC	Caucasian	Pyrosequencing	PB	1406/2506	1.00	8
Slattery	2009	USA	CRC	Mix	Taqman	PB	1577/1972	0.11	9
Randerson-Moor	2009a	UK	SC	Caucasian	PCR-RFLP	PB	1028/402	0.99	7
	2009b	UK	SC	Caucasian	PCR-RFLP	PB	299/560	0.20	8
Tworoger	2009a	USA	OC	Mix	Taqman	PB	1120/1158	0.11	7
	2009b	USA	OC	Mix	Taqman	PB	285/752	0.07	8
Anderson	2011	Canada	BC	Caucasian	PCR-RFLP	PB	1509/1590	0.06	8
Anic	2012	USA	Glioma	Caucasian	PCR-RFLP	HB	553/561	0.57	9
Bentley	2012	New Zealand	CRC	Caucasian	Taqman	PB	199/182	0.43	8
Rowland	2012	USA	PC	African-American	Taqman	PB	414/223	0.07	9
Hong	2012a	USA	BC	African-American	IlluminaGolden	PB	545/461	0.09	9
	2012b	USA	BC	Caucasian	IlluminaGolden	PB	381/382	0.89	9
Rowland	2013	USA	PC	Caucasian	Taqman	PB	1117/795	0.55	9
Gu	2014	China	EAC	Asian	LDR	HB	604/664	0.52	8
Peng	2014	China	HCC	Asian	SSP-PCR	HB	184/180	0.24	7
Iqbal	2015	Pakistan	BC	Asian	TARMS-PCR	HB	97/161	0.07	8

* All cases of cancer in each study were diagnosed by histology or pathology.A-A = African-American; BC = breast cancer; CRC = colorectal cancer; EAC = esophageal adenocarcinoma; HB = hospital based; HCC = hepatocellular carcinoma; HWE = Hardy–Weinberg equilibrium; LDR = ligation detection reaction method; NOS = the Newcastle–Ottawa Scale; OC = ovary cancer; PB = population based; PC = prostate cancer; PCR-RFLP = polymerase chain reaction and restriction fragment length polymorphism; SC = skin cancer; SSP-PCR = sequence-specific primers polymerase chain reaction; TARMS-PCR = tetraprimer amplification refractory mutation system polymerase chain reaction.

TABLE 2. Cdx-2 Polymorphism Genotype Distribution and Allele Frequency in Cases and Controls

	Genotype (N)												
	Case				Control				Case		Control		
First Author	Total	GG	GA	AA	Total	GG	GA	AA	G	A	G	A	MAF
Woodford-Richens 2001	49	35	11	3	51	40	11	0	81	17	91	11	0.17
John 2005	417	268	129	20	435	263	149	23	665	169	675	195	0.20
Han 2006	781	512	240	29	853	548	269	36	1264	298	1365	341	0.19
Mikhak 2007	688	406	246	36	689	425	224	40	1058	318	1074	304	0.23
Lurie 2007	70	44	21	5	145	95	44	6	109	31	234	56	0.22
Flügge 2007	256	104	128	24	256	104	128	24	336	176	336	176	0.34
Torkko 2008	444	282	131	31	488	323	148	17	695	193	794	182	0.22
Ochs-Balcom 2008	250	145	82	23	246	156	80	10	372	128	392	100	0.26
Theodoratou 2008	1996	1226	678	92	2037	1308	643	86	3130	862	3259	815	0.22
Abbas 2008	1406	888	465	53	2506	1701	795	10	2241	571	4197	815	0.20
Tworoger 2009a	1120	670	399	51	1158	746	356	56	1739	501	1848	468	0.23
Tworoger 2009b	285	179	92	14	752	496	220	36	450	120	1212	292	0.21
Randerson-Moor 2009a	1028	648	324	56	402	250	134	18	1620	436	634	170	0.21
Randerson-Moor 2009b	299	193	89	17	560	350	179	31	475	123	879	241	0.21
Slattery 2009	1577	957	515	105	1972	1241	632	99	2429	725	3114	830	0.23
Nic 2009	553	346	190	17	561	356	179	26	882	224	891	231	0.20
Anderson 2011	1509	969	456	84	1590	983	550	57	2394	624	2516	664	0.21
Hong 2012a	545	18	143	384	461	26	140	295	179	911	192	730	0.84
Hong 2012b	381	234	129	18	382	232	132	18	597	165	596	168	0.22
Bentley 2012	199	120	71	8	182	113	63	6	311	87	289	75	0.22
Rowland 2012	414	25	137	252	223	22	78	123	187	641	122	324	0.77
Rowland 2013	1117	706	347	64	795	481	271	43	1759	475	1233	357	0.21
Gu 2014	604	18	313	108	664	217	318	129	679	529	752	576	0.44
Peng 2014	184	63	92	29	180	61	94	25	218	150	216	144	0.41
Iqbal 2015	97	53	37	7	161	62	84	15	143	51	208	114	0.26

MAF = minor allele frequencies.

and the frequency of the minor allele distributed widely across the 25 eligible studies, ranging from 0.17 to 0.84. The average frequency of the minor allele was 0.28.

Main Results

Overall, our result suggested that the VDR Cdx-2 polymorphism was significant associated with increased cancer risk in the homozygote comparison (AA vs GG: OR = 1.23, 95% CI = 1.01–1.48, $P = 0.03$, Figure 2) and the allele comparison (A vs G: OR = 1.07, 95% CI = 1.01–1.13, $P = 0.01$). In the subgroup analysis by ethnicity, no significant correlation was observed between the Cdx-2 variation and cancer risk in Caucasians and Asians. However, significant association was found in American-Africans in 4 comparison models (AA vs GG: OR = 1.84, 95% CI = 1.19–2.85, $P = 0.006$; AA vs GG + GA: OR = 1.31, 95% CI = 1.07–1.61, $P = 0.01$; AA + AG vs GG: OR = 1.73, 95% CI = 1.12–2.65, $P = 0.01$; A vs G: OR = 1.32, 95% CI = 1.11–1.57, $P = 0.002$). When stratifying by source of controls, Cdx-2 polymorphism was detected to be significantly associated with an increased cancer risk in the following genetic models (AA vs GG: OR = 1.39, 95% CI = 1.12–1.72, $P = 0.003$; AA + AG vs GG: OR = 1.32, 95% CI = 1.09–1.60, $P = 0.004$; AA + GA vs GG: OR = 1.08, 95% CI = 1.03–1.14, $P = 0.002$; A vs G: OR = 1.10, 95% CI = 1.04–1.16, $P = 0.001$) in population-based case–control studies, whereas no statistical significance was found in hospital-based case–control studies. All comparisons are shown in Table 3.

In the cancer-specific analyses, there were 6 studies with 4327 cases and 4744 controls for colorectal cancer. The results showed significant correlation between Cdx-2 polymorphism and an increased risk of colorectal cancer in different comparison models (AA vs GG: OR = 1.30, 95% CI = 1.08–1.57, $P = 0.006$; AA vs GG + GA: OR = 1.27, 95% CI = 1.05–1.52, $P = 0.01$; AA + GA vs GG: OR = 1.12, 95% CI = 1.02–1.21, $P = 0.01$; A vs G: OR = 1.12, 95% CI = 1.04–1.20 $P = 0.002$). Furthermore, we identified 3 studies with 1475 cases and 2055 controls for ovarian cancer. The result showed significant association between the Cdx-2 polymorphism and ovarian cancer susceptibility in the dominant model (AA + GA vs GG: OR = 1.19, 95% CI = 1.04–1.37, $P = 0.01$) and the heterozygote comparison (GA vs GG: OR = 1.21, 95% CI = 1.05–1.41, $P = 0.01$) and the allele comparison (A vs G: OR = 1.13, 95% CI = 1.01–1.28, $P = 0.04$), but not in other genetic models.

There were 5 studies with 3080 cases and 2630 controls for prostate cancer, 5 studies with 3938 cases and 5100 controls for breast cancer and 3 studies with 2108 cases and 1815 controls for skin cancer (cutaneous melanoma, basal cell carcinoma, and squamous cell carcinoma), respectively. However, no statistical significance was found between the Cdx-2 polymorphism and prostate, breast or skin cancer susceptibility in any genetic model.

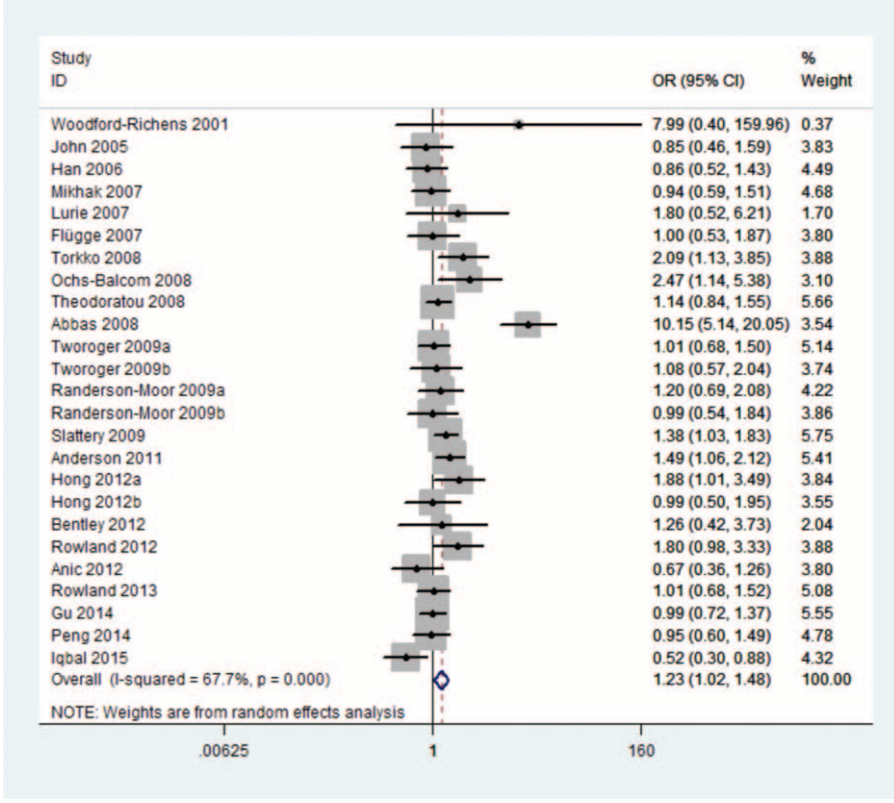

FIGURE 2. Forest plots of Cdx-2 polymorphism and cancer risk in overall population (AA vs GG).

Tests of Heterogeneity

As shown in Table 4, statistically significant heterogeneity was observed between trials of the following analyses using Q statistic. When the *P* value of the heterogeneity test was more than 0.1 ($P \geq 0.1$), a fixed-effects model was performed. Otherwise, the random-effects model was used.

Publication Bias

Begg's funnel plot and Egger's test were used to evaluate the publication bias. The Begg's funnel plots' shape seemed symmetrical (Figure 3). Therefore, there was no significant evidence for publication bias in our meta-analysis ($P = 0.45$).

TABLE 3. Meta-Analysis Results

	A vs G		AA vs GG		AA vs GA + GG		AA + GA vs GG		GA vs GG	
Comparisons	**OR (95% CI)**	***P***	**OR (95% CI)**	***P***	**OR (95% CI)**	***P***	**OR (95% CI)**	***P***	**OR (95% CI)**	***P***
Overall	1.07 (1.01–1.13)	**0.02**	1.23 (1.02–1.48)	**0.03**	1.18 (1.00–1.40)	0.05	1.05 (0.99–1.12)	0.10	1.04 (0.99–1.09)	0.11
Ethnicity										
Caucasian	1.04 (0.97–1.11)	0.30	1.31 (0.97–1.76)	0.07	1.32 (0.99–1.76)	0.06	1.03 (0.97–1.09)	0.36	0.99 (0.94–1.05)	0.82
A-A	1.32 (1.11–1.57)	**0.002**	1.84 (1.19–2.85)	**0.006**	1.31 (1.07–1.61)	**0.01**	1.73 (1.12–2.65)	**0.01**	1.51 (0.96–2.38)	0.08
Asian	0.97 (0.85–1.10)	0.64	0.86 (0.68–1.09)	0.21	0.84 (0.68–1.03)	0.10	0.87 (0.57–1.32)	0.51	1.11 (0.89–1.39)	0.36
Source of controls										
PB	1.10 (1.04–1.16)	**0.001**	1.39 (1.12–1.72)	**0.003**	1.32 (1.09–1.60)	**0.004**	1.08 (1.03–1.14)	**0.002**	1.05 (0.99–1.10)	0.09
HB	0.96 (0.88–1.05)	0.36	0.87 (0.72–1.06)	0.17	0.86 (0.72–1.03)	0.10	0.94 (0.78–1.13)	0.50	1.00 (0.88–1.13)	0.98
Cancer type										
CRC	1.12 (1.04–1.20)	**0.002**	1.30 (1.08–1.57)	**0.006**	1.27 (1.05–1.52)	**0.01**	1.12 (1.02–1.21)	**0.01**	1.09 (0.99–1.19)	0.07
PC	1.05 (0.92–1.20)	0.48	1.17 (0.93–1.48)	0.18	1.16 (0.95–1.41)	0.14	1.00 (0.89–1.12)	0.99	0.98 (0.87–1.10)	0.73
BC	1.06 (0.87–1.29)	0.54	1.69 (0.972–3.94)	0.23	1.69 (0.82–3.10)	0.17	1.00 (0.78–1.28)	0.98	0.98 (0.81–1.20)	0.86
OC	1.13 (1.04–1.28)	**0.04**	1.07 (0.77–1.48)	0.69	1.00 (0.73–1.38)	0.99	1.19 (1.04–1.37)	**0.01**	1.21 (1.05–1.41)	**0.01**
SC	0.96 (0.86–1.08)	0.53	1.00 (0.73–1.38)	0.99	1.03 (0.75–1.40)	0.87	0.94 (0.82–1.08)	0.41	0.94 (0.81–1.08)	0.36

A-A = African-American; BC = breast cancer; CI = confidence interval; CRC = colorectal cancer; HB = hospital based; OC = ovary cancer; OR = odds ratio; PB = population based; PC = prostate cancer; SC = skin cancer.

TABLE 4. Heterogeneity-Analysis Results

	A vs G			AA vs GG			AA vs GA + GG			AA + GA vs GG			GA vs GG		
Comparisons	I^2, %	*P*	EM	I^2, %	*P*	EM	I^2, %	*P*	EM	I^2, %	*P*	EM	I^2, %	*P*	EM
Overall	44	0.01	R	68	<0.001	R	67	<0.001	R	35	0.05	R	15	0.26	F
Ethnicity															
Caucasian	47	0.02	R	73	<0.001	R	73	<0.001	R	25	0.18	F	7	0.37	F
African-American	0	0.84	F	0	0.93	F	0	0.78	F	0	0.95	F	0	0.92	F
Asian	55	0.11	F	55	0.11	F	26	0.26	F	72	0.02	R	10	0.33	F
Source of controls															
PB	37	0.05	R	66	<0.001	R	65	<0.001	R	24	0.16	F	15	0.27	F
HB	15	0.32	F	30	0.22	F	13	0.33	F	51	0.09	R	26	0.25	F
Cancer type															
CRC	0	0.63	R	10	0.35	F	13	0.33	F	0	0.95	F	0	0.99	F
PC	51	0.08	R	48	0.11	F	32	0.21	F	47	0.11	F	36	0.19	F
BC	82	<0.001	R	92	<0.001	R	91	<0.001	R	80	0.001	R	61	0.04	R
OC	0	0.96	F	0	0.69	F	0	0.62	F	0	0.92	F	0	0.80	F
SC	0	0.89	F	0	0.69	F	0	0.67	F	0	0.96	F	0	0.96	F

BC = breast cancer; EM = effects model; F = fixed effects model; HB = hospital based; CRC = colorectal cancer; OC = ovary cancer; PB = population based; PC = prostate cancer; R = random effects model; SC = skin cancer.

Sensitivity Analysis

Sensitivity analysis was performed by sequentially omitting 1 individual study at a time, in order to reflect the influence of each study on the overall meta-analysis. As shown in Figure 4, sensitivity tests suggested that no single study greatly influenced the estimates of overall risk for the VDR Cdx-2 polymorphism, thus the results of our meta-analysis were stable.

DISCUSSION

According to the World Health Organization, an estimated 14.1 million new cancer cases and 8.2 million cancer-related deaths occurred in 2012.[36] Malignant tumors therefore become the leading cause of death worldwide. Biological as well as epidemiologic data suggest that vitamin D level could modulate the risk of some cancer and play a role in cancer prevention.[9] VDR can influence cancer predisposition through binding to vitamin D. Polymorphisms of the VDR gene promoter region have been associated with several forms of cancer.[37,38] Although a large number of previous studies have been published to investigate the association between Cdx-2 polymorphism and cancer risk, these studies always reported mixed results. To further precisely evaluate the association, the current meta-analysis is performed on all eligible case–control studies.

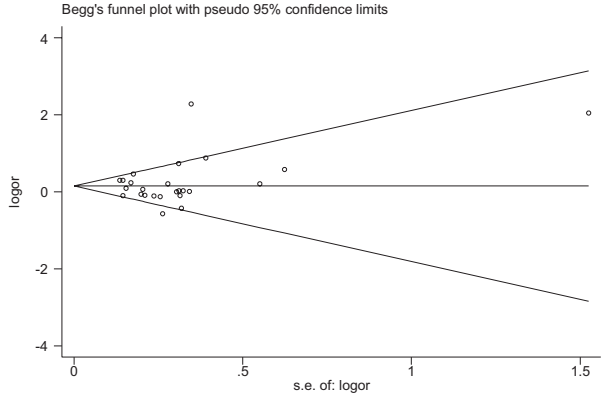

FIGURE 3. Begg's funnel plot assessing evidence of publication bias from the eligible studies.

In the present meta-analysis, we have included a total of 16,269 cases and 17,749 controls from 25 eligibility studies and found that Cdx-2 polymorphism significant associated with overall cancer risk. The results showed significant association for the Cdx-2 variant and cancer risk under the homozygote and allele models, respectively. The data indicated that the Cdx-2 A allele may exert substantial biological impact on the development of cancer. Dysfunction of vitamin D metabolism pathways may be involved in the carcinogenesis.

Because of different gene–environment interplays may exist in different ethnicity backgrounds, we conducted an ethnicity-based subgroup analysis and the results demonstrated that Cdx-2 was significant association with an increased risk of cancer in the following comparison models (AA vs GG: OR = 1.84, 95% CI = 1.19–2.85; AA vs GG + GA: OR = 1.31, 95% CI = 1.07–1.61; AA + GA vs GG: OR = 1.73, 95% CI = 1.12–2.65) in African-Americans. However, no significant association was observed between the Cdx-2 variant and Caucasians or Asians. Fang et al[39] revealed that the Cdx-2 A allele occurs more commonly in African (74%) populations than among the Caucasians (19%). Therefore, we suggested that these data should be explained with caution considering the heterogeneity of the ethnicity subgroup. Dietary habits, sun exposure and genetic backgrounds can influence the correlation between polymorphisms and cancer susceptibility.

In the cancer-specific analyses, we were able to provide a comprehensive evaluation of the role of the Cdx-2 polymorphism in several different cancers susceptibility. Our meta-analysis included 7 new independent studies, based on prostate cancer,[30] colorectal cancer,[23] glima,[26] esophageal cancer,[31] hepatocellular carcinoma,[32] and breast cancer,[33] which were not included in a previous meta-analysis.[34] Furthermore, 2 different studies for ovarian carcinoma and prostate cancer were excluded in our study because the HWE was insufficient.[16,21] In another meta-analysis that discussed the

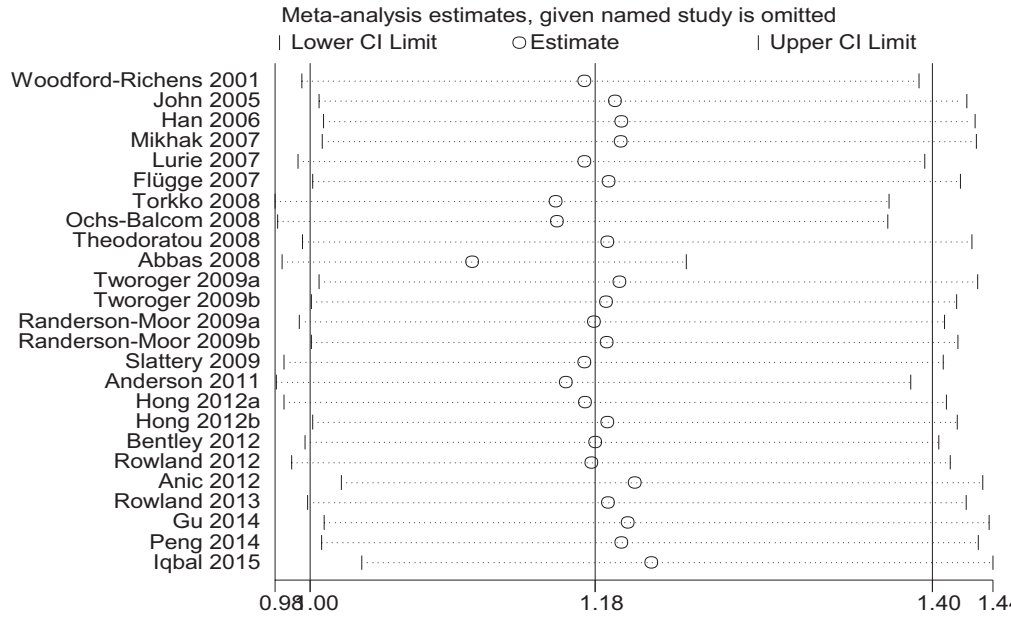

FIGURE 4. Sensitivity analysis of association between Cdx-2 polymorphism and cancer.

association between Cdx2 polymorphism and breast cancer susceptibility, significance was only detected on Africans.[40] Our study included more eligible researches performed in Asians. The results also showed no significant association between Cdx-2 polymorphism and breast cancer susceptibility in overall populations. In the subgroup analysis by ethnicity, significant correlation was observed between Cdx-2 polymorphism and cancer risk in African-Americans but not in Caucasians or Asians. More notable was that our study included more independent studies involved other different cancer types,[30–33] and the results provided stronger evidence that Cdx-2 polymorphism might associate with cancer risk in Africans.

Our meta-analyses showed that the Cdx-2 AA genotype significantly increased colorectal cancer risk by 30% and 27% versus GG and (GG + GA), respectively. In addition, this current meta-analysis showed that GG heterozygote genotype had a 21% and 19% decreased risk of ovary cancer than GA homozygote and (AA + GA), respectively. Our results suggested that Cdx-2 GG genotype may contribute to a protective effect in ovary cancer and it need to be confirmed by studies with larger sample sizes. However, no significant associations were found among studies of skin, breast, and prostate cancer in any genetic models. According our meta-analysis results, the inconsistent results might be due to different functional mechanisms of vitamin D in different cancer tissues. The results also suggested that the influence of the VDR genetic polymorphism may be ambiguous due to the presence of other factors, such as sun exposure, vitamin D intake, and involvement, in different cancers.

There were several limitations that should be noted in this meta-analysis. First, our study was lack of original information of Cdx-2 gene transcription and expression. The Cdx-2 polymorphism located in the promoter region of VDR gene might affect the transcription and expression of VDR, which might further influence the absorption of vitamin D.[4] Vitamin D exerted functions in the immune, neural, and endocrine systems and were involved in the regulation of tumor growth.[41] Second, our meta-analysis was based on unadjusted estimates so that we could not assess the risk of cancer according to stratification of age, diet, calcium and vitamin D intake, UV exposure, and other risk factors of cancer. The lack of such data for the meta-analysis may cause confounding bias. Third, there were only 2 studies on Africans and 3 studies on Asians included in this meta-analysis, thus the conclusion should be interpreted with caution at overall population. Fourth, for some cancers there was only 1 study, which may lead to heterogeneity in quantitative analysis. In addition, the potential influence on genotype-cancer associations by environmental factors is worthy of consideration.

CONCLUSIONS

This meta-analysis provides an updated and comprehensive meta-analysis about the role of the VDR Cdx-2 polymorphism in cancer susceptibility. Our results showed that the Cdx-2 polymorphism is associated with an increased risk of some cancers. Further studies were needed to confirm the prediagnostic effect of Cdx-2 gene polymorphism in carcinogenesis.

REFERENCES

1. Holick MF. Vitamin D deficiency. *N Engl J Med.* 2007;357:266–281.
2. Vuolo L, Di Somma C, Faggiano A, et al. Vitamin D and cancer. *Front Endocrinol (Lausanne).* 2012;3:58.
3. Uitterlinden AG, Fang Y, Van Meurs JB, et al. Genetics and biology of vitamin D receptor polymorphisms. *Gene.* 2004;338:143–156.
4. Arai H, Miyamoto KI, Yoshida M, et al. The polymorphism in the caudal-related homeodomain protein Cdx-2 binding element in the human vitamin D receptor gene. *J Bone Miner Res.* 2001;16:1256–1264.
5. Pharoah PD, Dunning AM, Ponder BA, et al. Association studies for finding cancer-susceptibility genetic variants. *Nat Rev Cancer.* 2004;4:850–860.

6. Churilla TM, Lesko SL, Brereton HD, et al. Serum vitamin D levels among patients in a clinical oncology practice compared to primary care patients in the same community: a case-control study. *BMJ Open.* 2011;1:e000397.
7. Churilla TM, Brereton HD, Klem M, et al. Vitamin D deficiency is widespread in cancer patients and correlates with advanced stage disease: a community oncology experience. *Nutr Cancer.* 2012;64:521–525.
8. van den Bemd GJ, Chang GT. Vitamin D and vitamin D analogs in cancer treatment. *Curr Drug Targets.* 2002;3:85–94.
9. Luong K, Nguyen LT. The beneficial role of vitamin D and its analogs in cancer treatment and prevention. *Crit Rev Oncol Hematol.* 2010;73:192–201.
10. Kupfer SS, Anderson JR, Ludvik AE, et al. Genetic associations in the vitamin D receptor and colorectal cancer in African Americans and Caucasians. *PLoS ONE.* 2011;6:e26123.
11. Ditsch N, Toth B, Mayr D, et al. The association between vitamin D receptor expression and prolonged overall survival in breast cancer. *J Histochem Cytochem.* 2012;60:121–129.
12. Woodford-Richens KL, Halford S, Rowan A, et al. CDX2 mutations do not account for juvenile polyposis or Peutz-Jeghers syndrome and occur infrequently in sporadic colorectal cancers. *Br J Cancer.* 2001;84:1314–1316.
13. John EM, Schwartz GG, Koo J, et al. Sun exposure, vitamin D receptor gene polymorphisms, and risk of advanced prostate cancer. *Cancer Res.* 2005;65:5470–5479.
14. Flugge J, Krusekopf S, Goldammer M, et al. Vitamin D receptor haplotypes protect against development of colorectal cancer. *Eur J Clin Pharmacol.* 2007;63:997–1005.
15. Han J, Colditz GA, Hunter DJ. Polymorphisms in the MTHFR and VDR genes and skin cancer risk. *Carcinogenesis.* 2007;28:390–397.
16. Lurie G, Wilkens LR, Thompson PJ, et al. Vitamin D receptor gene polymorphisms and epithelial ovarian cancer risk. *Cancer Epidemiol Biomarkers Prev.* 2007;16:2566–2571.
17. Mikhak B, Hunter DJ, Spiegelman D, et al. Vitamin D receptor (VDR) gene polymorphisms and haplotypes, interactions with plasma 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D, and prostate cancer risk. *Prostate.* 2007;67:911–923.
18. Abbas S, Nieters A, Linseisen J, et al. Vitamin D receptor gene polymorphisms and haplotypes and postmenopausal breast cancer risk. *Breast Cancer Res.* 2008;10:R31.
19. Ochs-Balcom HM, Cicek MS, Thompson CL, et al. Association of vitamin D receptor gene variants, adiposity and colon cancer. *Carcinogenesis.* 2008;29:1788–1793.
20. Theodoratou E, Farrington SM, Tenesa A, et al. 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.* 2008;123:2170–2179.
21. Torkko KC, van Bokhoven A, Mai P, et al. VDR and SRD5A2 polymorphisms combine to increase risk for prostate cancer in both non-Hispanic White and Hispanic White men. *Clin Cancer Res.* 2008;14:3223–3229.
22. Randerson-Moor JA, Taylor JC, Elliott F, et al. Vitamin D receptor gene polymorphisms, serum 25-hydroxyvitamin D levels, and melanoma: UK case-control comparisons and a meta-analysis of published VDR data. *Eur J Cancer.* 2009;45:3271–3281.
23. Slattery ML, Wolff RK, Curtin K, et al. Colon tumor mutations and epigenetic changes associated with genetic polymorphism: insight into disease pathways. *Mutat Res.* 2009;660:12–21.
24. Tworoger SS, Gates MA, Lee IM, et al. Polymorphisms in the vitamin D receptor and risk of ovarian cancer in four studies. *Cancer Res.* 2009;69:1885–1891.
25. Anderson LN, Cotterchio M, Cole DE, et al. Vitamin D-related genetic variants, interactions with vitamin D exposure, and breast cancer risk among Caucasian women in Ontario. *Cancer Epidemiol Biomarkers Prev.* 2011;20:1708–1717.
26. Anic GM, Thompson RC, Nabors LB, et al. An exploratory analysis of common genetic variants in the vitamin D pathway including genome-wide associated variants in relation to glioma risk and outcome. *Cancer Causes Control.* 2012;23:1443–1449.
27. Bentley RW, Keown DA, Gearry RB, et al. Vitamin D receptor polymorphisms in colorectal cancer in New Zealand: an association study. *N Z Med J.* 2012;125:47–51.
28. Rowland GW, Schwartz GG, John EM, et al. Calcium intake and prostate cancer among African Americans: effect modification by vitamin D receptor calcium absorption genotype. *J Bone Miner Res.* 2012;27:187–194.
29. Yao S, Zirpoli G, Bovbjerg DH, et al. Variants in the vitamin D pathway, serum levels of vitamin D, and estrogen receptor negative breast cancer among African-American women: a case-control study. *Breast Cancer Res.* 2012;14:R58.
30. Rowland GW, Schwartz GG, John EM, et al. Protective effects of low calcium intake and low calcium absorption vitamin D receptor genotype in the California Collaborative Prostate Cancer Study. *Cancer Epidemiol Biomarkers Prev.* 2013;22:16–24.
31. Gu H, Wang X, Zheng L, et al. Vitamin D receptor gene polymorphisms and esophageal cancer risk in a Chinese population: a negative study. *Med Oncol.* 2014;31:827.
32. Peng Q, Yang S, Lao X, et al. Association of single nucleotide polymorphisms in VDR and DBP genes with HBV-related hepatocellular carcinoma risk in a Chinese population. *PLoS ONE.* 2014;9:e116026.
33. Iqbal MU, Khan TA, Maqbool SA. Vitamin D receptor Cdx-2 polymorphism and premenopausal breast cancer risk in southern Pakistani patients. *PLoS ONE.* 2015;10:e0122657.
34. Huang J, Ma Y, Wang H, et al. The Cdx-2 polymorphism in the VDR gene is associated with increased risk of cancer: a meta-analysis. *Mol Biol Rep.* 2013;40:4219–4225.
35. Stang A. Critical evaluation of the Newcastle–Ottawa scale for the assessment of the quality of nonrandomized studies in meta-analyses. *Eur J Epidemiol.* 2010;25:603–605.
36. Ferlay J, Soerjomataram I, Dikshit R, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer.* 2015;136:E359–E386.
37. Norman AW. Minireview: vitamin D receptor: new assignments for an already busy receptor. *Endocrinology.* 2006;147:5542–5548.
38. Friedrich M, Rafi L, Mitschele T, et al. Analysis of the vitamin D system in cervical carcinomas, breast cancer and ovarian cancer. *Recent Results Cancer Res.* 2003;164:239–246.
39. Fang Y, van Meurs JB, Bergink AP, et al. Cdx-2 polymorphism in the promoter region of the human vitamin D receptor gene determines susceptibility to fracture in the elderly. *J Bone Miner Res.* 2003;18:1632–1641.
40. Zhou ZC, Wang J, Cai ZH, et al. Association between vitamin D receptor gene Cdx2 polymorphism and breast cancer susceptibility. *Tumour Biol.* 2013;34:3437–3441.
41. Haussler MR, Whitfield GK, Haussler CA, et al. The nuclear vitamin D receptor: biological and molecular regulatory properties revealed. *J Bone Miner Res.* 1998;13:325–349.