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Original Article

GC Glu416Asp and Thr420Lys polymorphisms contribute to gastrointestinal cancer susceptibility in a Chinese population

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Abstract: Vitamin D has potent anticancer properties, especially against gastrointestinal cancers. Group-specific component (GC), a key member of vitamin D pathway proteins, could bind to and transport vitamin D to target organs. As a polymorphic protein, two common coding single nucleotide polymorphisms (SNP) [Glu416Asp (rs7041) and Thr420Lys (rs4588)] were identified in its gene. These SNPs have been associated to circulating vitamin D levels and several cancer risks in different populations. However, there is no report on their role in gastrointestinal cancer development among Chinese to date. Therefore, we examined the association between these variants and risk of gastrointestinal cancers in a case-control cohort including 964 patients with four gastrointestinal cancers (hepatocellular carcinoma, esophageal cancer, gastric cancer and colorectal cancer) and 1187 controls. Odds ratios and 95% confidence intervals were estimated by logistic regression. We found that *GC* Thr420Lys polymorphism has significant impact on the risk of developing gastrointestinal cancers, especially colorectal cancer. Additionally, subjects who carrying *GC* Asp_{416}-Lys_{420} haplotype, which contains the at-risk 420Lys allele, also showed significantly increased risk to develop gastrointestinal cancers. In conclusion, our study demonstrated that common genetic variants and haplotypes in *GC* may influence individual susceptibility to gastrointestinal cancers in Chinese population.

Keywords: Group-specific component, single nucleotide polymorphism, gastrointestinal cancer, susceptibility

Introduction

Vitamin D has potent anticancer properties, especially against gastrointestinal cancers. Reduced risk for colorectal cancer (CRC) was firstly correlated to vitamin D levels *in vivo* in 1980 [1]. This inverse relationship between vitamin D intake or circulating 25-hydroxyvitamin D [25 (OH)D], a biomarker of vitamin D status and CRC was supported in both cohort studies [2-5] and case-control studies [6-8], although the association did not reach statistical significance in all reports. Similar inverse correlation was observed between vitamin D and esophageal cancer (EC) risk [5, 9]. Although inconsistent results on relationship between vitamin D and gastric cancer (GCa) were reported [5, 10], Ikezaki S et al did show that 24R,25-dihydroxyvitamin D3 (a vitamin D3 derivative) exerts chemopreventive effects on glandular stomach carcinogenesis in rats [11]. For hepatocellular carcinoma (HCC), there are few epidemiology studies on role of vitamin D in oncogenesis of liver. However, 1,25-dihydroxyvitamin D3 (another vitamin D3 derivative) exhibited significantly anti-HCC activities *ex vivo* and *in vivo* [12-14], indicating the potential protective role of vitamin D in HCC development.

As a key protein of vitamin D pathway, group-specific component (GC, also known as vitamin D-binding protein) could bind to vitamin D and its plasma metabolites and transport them to target tissues. In addition, GC can be converted into a macrophage activating factor (GcMAF) by the stepwise action of β-galactosidase of B cells and sialidase of T cells [15]. During inflammation, GcMAF could stimulate phagocytotic activ-

ity of macrophages [16]. Interestingly, GcMAF also shown antitumor activities in mice [17] and may be a potential immunotherapeutic reagent for metastatic breast cancer [18]. All these evidence suggest that GC may play an important part during carcinogenesis, alone or in a combination manner with vitamin D.

The *GC* gene, which coding human GC, is polymorphic. There are two common non-synonymous coding single nucleotide polymorphisms (SNP) [Glu416Asp (rs7041) and Thr420Lys (rs4588)] in this gene. Glu416Asp and Thr420Lys have been shown to alter plasma concentrations of 25(OH)D in candidate gene studies [19, 20]. Recently, genome wide association studies (GWAS) also shown that *GC* rs2282679, which is in strong linkage disequilibrium (LD) with Glu416Asp, was associated with serum 25(OH)D levels [21, 22]. In the previous reports, selected genetic variants in the *GC* gene, including Glu416Asp and Thr420Lys, have been investigated in breast cancer [23-25], prostate cancer [26], CRC [27] and basal cell carcinomas [28]. However, to date, there is no systematic evaluation on how common Glu416Asp and Thr420Lys SNPs are involved in development of gastrointestinal cancers in Chinese. Therefore, we specifically examined whether these two non-synonymous SNPs are associated with development of four gastrointestinal cancers (EC, HCC, GCa and CRC) in the current study. In addition, the role of *GC* haplotypes in development of gastrointestinal cancers was also evaluated.

Materials and methods

Study cohorts

This study consisted of 964 patients with gastrointestinal cancers (237 HCC cases, 289 EC cases, 192 GCa cases and 246 CRC cases) and 1187 healthy controls. HCC, EC, and GCa patients were consecutively recruited between January 2009 and July 2011, at Huaian No. 2 Hospital, Huaian, Jiangsu Province, China. CRC patients were recruited between January 2009 and October 2011, at Huaian No. 2 Hospital. Controls were cancer-free individuals selected from a community cancer-screening program for early detection of cancer conducted during the same time period as the patients were collected. Controls were matched to patients on age (± 5 years) and sex. All subjects were ethnic Han Chinese. At recruitment, informed consent was obtained from each subject and each participant was then interviewed to collect the information on demographic characteristics and lifetime history of tobacco use. This study was approved by the institutional Review Board of Huaian No. 2 Hospital.

Polymorphism genotyping

GC Asp416Glu and Thr420Lys genotypes were examined using PCR-based restriction fragment length polymorphism (RFLP). In PCR-RFLP genotyping, the primers used for amplifying DNA segments containing either *GC* Asp416Glu or Thr420Lys were 5′-AGA TCT GAA ATG GCT ATTAT TTT GCA-3′/5′-TTG CCA GTT CCG TGG GTG AG-3′ or 5′-TCT GAA ATG GCT ATT ATT TTG CAT T-3′/5′-TGT TAA CCA GCT TTG CCA GTT AC-3′, respectively. The underline nucleotides are mismatch nucleotides introduced to create an *Eco147*I site or an *HpyCH4*IV site. Restriction enzyme *Eco147*I (Fermentas) or *HpyCH4*IV (New England Biolabs) was used to distinguish *GC* Asp416Glu or Thr420Lys genotypes, respectively. A 15% random sample was reciprocally tested by direct sequencing, and the reproducibility was 99.9%.

Statistics

Pearson's χ^2 test was used to examine the differences in demographic variables, smoking status and genotype distributions of *GC* Asp416Glu and Thr420Lys polymorphisms between patients and controls. Associations between genotypes and risk of the development of gastrointestinal cancers were estimated by odds ratios (ORs) and their 95% confidence intervals (95% CIs) computed using unconditional logistic regression model. Smokers were considered current smokers if they smoked up to 1 year before the date of cancer diagnosis or if they smoked up to 1 year before the date of the interview for control subjects. Subjects who never smoked or smoked less than 1 year before the date of cancer diagnosis for case patients or the date of interview for control subjects were defined as nonsmokers. All ORs were adjusted for age, sex, or smoking status, where it was appropriate. A P value of less than 0.05 was used as the criterion of statistical significance, and all statistical tests were two-sided. All abovementioned analyses were performed with SPSS software (version 16.0). Haplo.stats [29] was also

Table 1. Distribution of selected characteristics among gastrointestinal cancer patients and controls

Variable	Hepatocellular carcinoma		Esophageal cancer		Gastric cancer		Colorectal cancer	
	Cases/ Control	*P*[a]	Cases/ Control	*P**	Cases/ Control	*P**	Cases/ Control	*P**
	237/315		289/337		192/204		246/331	
Age (year)		0.702		0.897		0.209		0.377
≤59	121/166		155/179		99/118		134/168	
>59	116/149		134/158		93/86		112/163	
Sex		0.587		0.616		0.481		0.420
Male	189/257		204/244		138/153		185/239	
Female	48/58		85/93		54/51		61/92	
Smoking status		<0.001		<0.001		<0.001		<0.001
No	83/177		67/169		51/114		81/183	
Yes	154/138		222/168		141/90		165/148	

*Two-sided χ^2 test

used to assess the relative effects of *GC* haplotypes on gastrointestinal cancers and adjusted for sex, age, and smoking status.

Results

Subject characteristics

As shown in **Table 1**, there were no statistically significant differences in the distributions of sex, age and smoking status between patients and controls, suggesting that the frequency matching was adequate. However, smokers were over-represented in gastrointestinal cancer patients compared with controls (all $P < 0.01$).

Genotype distributions of GC Asp416Glu and Thr420Lys polymorphisms

Genotyping results are shown in **Table 2**. The allele frequencies for *GC* 416Glu and 420Lys, respectively, were 0.281 and 0.301 in healthy controls as well as 0.283 and 0.336 in gastrointestinal cancer patients. All observed *GC* Asp416Glu and Thr420Lys genotype frequencies in both controls and patients conform to Hardy-Weinberg equilibrium (HWE) ($P_{HWE} > 0.05$), except *GC* Asp416Glu in the GCa case-control cohort ($P_{HWE} = 0.04$). Linkage disequilibrium analysis showed that these two SNPs are in strong linkage, with $D' = 1.00$ and $r^2 = 0.18$. Distributions of these *GC* Asp416Glu and Thr420Lys genotypes were then compared among patients and controls. Frequencies of 416Asp/Asp, Asp/Glu and Glu/Glu genotypes among all gastrointestinal cancer patients were not significantly different from those among controls ($\chi^2 = 0.500$, $P = 0.779$; degrees of freedom = 2). Significant difference was observed for 420Thr/Thr, Thr/Lys and Lys/Lys genotypes between gastrointestinal cancer patients and controls ($\chi^2 = 10.38$, $P = 0.006$; degrees of freedom = 2). In the stratified analyses, frequencies of *GC* 420Thr/Thr, Thr/Lys and Lys/Lys genotypes were significantly different among CRC patients and controls ($\chi^2 = 15.65$, $P = 0.0004$; degrees of freedom = 2), with the Lys/Lys variant being more prevalent in patients than in controls (13.4% vs. 4.5%).

Association between GC Asp416Glu and Thr420Lys polymorphisms and gastrointestinal cancer risks

No significant association between *GC* Asp416Glu or Thr420Lys SNPs and EC, HCC or GCa risk was observed (P_{treand} of EC = 0.232 for Asp416Glu or 0.616 for Thr420Lys; P_{treand} of HCC = 0.920 for Asp416Glu or 0.356 for Thr420Lys; P_{treand} of GCa = 0.310 for Asp416Glu or 0.249 for Thr420Lys) (**Table 2**). However, there was a significant correlation between the *GC* Thr420Lys genetic variant and CRC risk ($P_{treand} = 0.0009$) (**Table 2**). In detail, carriers of *GC* 420Lys/Lys genotype shown significantly elevated risk to develop CRC compared with 420Thr/Thr carriers (OR = 3.41, 95% CI = 1.85-6.57, $P = 1.01\times10^{-4}$). Although increased risk in carriers of 420Thr/Lys genotype was also found compared with 420Thr/Thr carri-

Table 2. GC genotype frequencies among patients and controls and their association with risk of gastrointestinal cancers

Cancer type	Asp416Glu (rs7041 T/G)				Thr420Lys (rs4588 C/A)			
	Genotype	Cases/ Controls[b]	OR[a] (95% CI)	P	Genotype	Cases/ Controls[b]	OR[a] (95% CI)	P
Hepatocellular carcinoma		237/315				237/315		
	Asp/Asp	117/152	Reference		Thr/Thr	101/142	Reference	
	Asp/Glu	98/139	1.07 (0.75-1.53)	0.697	Thr/Lys	111/148	1.05 (0.73-1.49)	0.808
	Glu/Glu	22/24	0.88 (0.64-1.21)	0.432	Lys/Lys	25/25	1.17 (0.86-1.60)	0.310
	P_{trend}[c]		0.920		P_{trend}[c]		0.356	
Esophageal cancer		289/337				289/337		
	148	148/188	Reference		Thr/Thr	148/159	Reference	
	119	119/128	0.82 (0.57-1.16)	0.260	Thr/Lys	108/144	0.78 (0.55-1.12)	0.181
	22	22/21	1.00 (0.69-1.44)	0.997	Lys/Lys	33/34	0.96 (0.72-1.28)	0.769
	P_{trend}[c]		0.232		P_{trend}[c]		0.616	
Gastric cancer		192/204				192/204		
	Asp/Asp	98/99	Reference		Thr/Thr	74/88	Reference	
	Asp/Glu	86/89	1.11 (0.71-1.73)	0.663	Thr/Lys	89/92	1.24 (0.78-1.99)	0.367
	Glu/Glu	8/16	1.45 (0.89-2.36)	0.133	Lys/Lys	29/24	1.25 (0.89-1.75)	0.200
	P_{trend}[c]		0.310		P_{trend}[c]		0.249	
Colorectal cancer		246/331				246/331		
	Asp/Asp	123/171	Reference		Thr/Thr	113/182	Reference	
	Asp/Glu	107/132	0.86 (0.65-1.24)	0.472	Thr/Lys	100/134	1.35 (0.92-1.71)	0.269
	Glu/Glu	16/28	1.28 (0.67-2.48)	0.484	Lys/Lys	33/15	3.41 (1.85-6.57)	1.01×10^{-4}
	P_{trend}[c]		0.956		P_{trend}[c]		0.0009	
Total		964/1187				964/1187		
	Asp/Asp	486/610	Reference		Thr/Thr	436/571	Reference	
	Asp/Glu	410/488	0.97 (0.83-1.13)	0.682	Thr/Lys	408/518	1.07 (0.92-1.25)	0.383
	Glu/Glu	68/89	1.02 (0.88-1.17)	0.841	Lys/Lys	120/98	1.15 (1.02-1.30)	0.020
	P_{trend}[c]		0.845		P_{trend}[c]		0.013	

[a]Data were calculated by unconditional logistic regression, adjusted for age, sex, and smoking status where it was appropriate. For all cancer together, the analyses were also adjusted for age, sex, and smoking status; [b]Number of subjects; [c]Tests for trend of odds were 2-sided and based on likelihood ratio tests assuming a multiplicative model.

Table 3. Distribution of *GC* haplotypes frequencies among patients and controls and their association with gastrointestinal cancers

Haplotypes	No. of chromosomes (%)		OR[a] (95% CI)	P[b]
	Patients	Controls		
Huaian cohort				
Asp_{416}-Thr_{420}	790 (41.0)	900 (37.9)	Reference	
Glu_{416}-Thr_{420}	542 (28.1)	669 (28.2)	1.11 (0.95-1.27)	0.273
Asp_{416}-Lys_{420}	594 (30.8)	802 (33.8)	1.22 (1.04-1.39)	0.015
Glu_{416}-Lys_{420}	2 (0.1)	2 (0.1)	NC	NC

NC, not calculated. [a]Adjusted for sex, age, and smoking; [b]After 1000 permutation test.

Table 4. Distribution of *GC* haplotypes frequencies among patients and controls and their association with colorectal cancer

Haplotypes	No. of chromosomes (%)		OR[a] (95% CI)	*P*[b]
	Patients	Controls		
Huaian cohort				
Asp_{416}-Thr_{420}	225 (45.8)	242 (36.5)	Reference	
Glu_{416}-Thr_{420}	130 (26.5)	194 (29.3)	1.43 (1.08-1.94)	0.023
Asp_{416}-Lys_{420}	136 (27.7)	226 (34.2)	1.57 (1.19-2.17)	0.002
Glu_{416}-Lys_{420}	0 (0.0)	0 (0.0)	NC	NC

NC, not calculated. [a]Adjusted for sex, age, and smoking; [b]After 1000 permutation test.

ers, the association did not reach statistical significance (OR = 1.35, 95% CI = 0.92-1.71, P = 0.269). No such association existed between CRC and *GC* Asp416Glu SNP (P_{treand} = 0.956) (**Table 2**). In the combined analysis of all gastrointestinal cancer patients and controls, we found that a 1.15-fold increased risk to develop gastrointestinal cancers in subjects carrying *GC* 420Lys/Lys genotype compared with 420Thr/Thr carriers (95% CI = 1.02-1.30, P = 0.020) after adjustment of age, sex, and smoking status (**Table 2**). However, there was no significant association between *GC* Asp416Glu polymorphism and gastrointestinal cancer risk (**Table 2**). We also examined whether there are gene-smoking interactions, but the results were negative (data not shown).

GC Asp416Glu and Thr420Lys haplotypes and gastrointestinal cancer risks

Haplotype analyses shown that, compared with *GC* Asp416-Thr420 haplotype, a significantly increased gastrointestinal cancer risk was observed in carriers of Asp416-Lys420 haplotype (OR = 1.22, 95% CI = 1.04-1.39, P = 0.015). However, there was no significant association between *GC* Glu416-Thr420 haplotype and gastrointestinal cancer risk (OR = 1.11, 95% CI = 0.95-1.27, P = 0.273) (**Table 3**). Considering the haplotype frequency of Glu416-Lys420 was only 0.1% in cases or controls, we did not calculate its OR and 95% CI (**Table 3**). For CRC, compared with *GC* Asp416-Thr420 haplotype, a significantly increased gastrointestinal cancer risk was observed in carriers of Asp416-Lys420 haplotype (OR = 1.57, 95% CI = 1.19-2.17, P = 0.002) or Glu416-Thr420 haplotype and gastrointestinal cancer risk (OR = 1.43, 95% CI = 1.08-1.94, P = 0.023) (**Table 4**).

Discussion

In this study, we examined whether *GC* Asp416Glu or Thr420Lys genetic polymorphisms are associated with risk of developing gastrointestinal cancers. On the basis of analysis of a Chinese Han case-control cohort (964

incident patients with gastrointestinal cancers and 1187 control subjects), we demonstrated that *GC* Thr420Lys polymorphism has significant impact on the risk of developing gastrointestinal cancers, especially on CRC oncogenesis. Additionally, subjects who carrying *GC* Asp416-Lys420 haplotype, which contains the *GC* at-risk 420Lys allele, also shown significantly increased risk to develop gastrointestinal cancers, especially CRC.

GC is a serum glycoprotein that belongs to the albumin family. As a key member in vitamin D metabolism pathway, GC can bind to 25(OH)D and other blood vitamin D sterol metabolites and transports them in circulation to target organs, including gastrointestinal organs. It was found that Glu416Asp and Thr420Lys could alter plasma concentrations of 25(OH)D [19, 20]. Sinotte M et al found that the plasma 25 (OH)D concentration of *GC* 420Lys/Lys carriers was significantly lower than that of 420Thr/Thr carriers (Crude mean ± SE = 59.0 ± 2.4 vs. 67.2 ± 1.0, P = 0.0016; adjusted mean ± SE = 58.4 ± 2.0 vs. 67.2 ± 0.9, P < 0.0001) [19]. Similar results were observed in the Insulin Resistance Atherosclerosis Family Study [20]. *GC* Thr420Lys was associated with levels of 25(OH) D and 1,25(OH)2D in both Hispanics and African Americans participants at all three study centers [20]. These results suggest that the increased risk of *GC* 420Lys/Lys carriers to develop gastrointestinal cancers (especially CRC) may be due to their low circulating 25(OH)D levels compared to 420Thr/Thr carriers, since clinically deficient 25(OH)D levels have been associated to increased cancer risk. In addition, the activation of the vitamin D receptor pathway was correlated to increased endocytotic uptakes of the GC-25(OH)D-complex to cancer cells, which in turn leads to anticarcinogenic action of vitamin D [30]. Therefore, it is biologically plausible that subjects carrying 420Lys/Lys genotype or Asp416-Lys420 haplotype have a lower uptake ability of GC-25(OH)D complex from circulation and, thus, a increased gastrointestinal cancer risk.

In the previous reports, three common phenotypic alleles (haplotypes) have been identified in the GC protein (Gc1s: Glu416-Thr420, Gc1f: Asp416-Thr420, and Gc2: Asp416-Lys420) [31, 32]. These GC haplotypes have been associated with differences in serum 25(OH)D concentration and affinity of GC to vitamin D metabolites. Compared with Asp416-Lys420 haplotype, *GC* Glu416-Thr420 and Asp416-Thr420 haplotypes were associated with higher levels of serum 25 (OH)D concentration as well as higher affinity of GC to vitamin D metabolites [32-34]. Consistent with these previous findings, we also identified the same three common haplotypes in Chinese population and found that Asp416-Lys420 haplotype was associated with significantly increased gastrointestinal cancer risk. This observation is biologically plausible since the Asp416-Lys420 haplotype carrier may be in vitamin D deficient status and their gastrointestinal cells more easily undergo malignant transformation compared to carriers of these other two common haploptypes.

In summary, our study demonstrates that *GC* Thr420Lys genetic polymorphism and Asp416-Lys420 haplotype are associated with increased risk of gastrointestinal cancers in Chinese Han population. However, as a hospital-based case-control study, our study may have selection bias and/or systematic error because the cases were recruited from a hospital and the controls were recruited from the community. Therefore, independent studies are needed to validate our findings.

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References

[1] Garland CF, Garland FC. Do sunlight and vitamin D reduce the likelihood of colon cancer? Int J Epidemiol 1980; 9: 227-231.

[2] Bostick RM, Potter JD, Sellers TA, McKenzie DR, Kushi LH and Folsom AR. Relation of calcium, vitamin D, and dairy food intake to incidence of colon cancer among older women. The Iowa Women's Health Study. Am J Epidemiol 1993; 137: 1302-1317.

[3] Martínez ME, Marshall JR, Sampliner R, Wilkinson J and Alberts DS. Calcium, vitamin D, and risk of adenoma recurrence (United States). Cancer Causes Control 2002; 13: 213-220.

[4] Martínez ME, Giovannucci EL, Colditz GA, Stampfer MJ, Hunter DJ, Speizer FE, Wing A and Willett WC. Calcium, vitamin D, and the occurrence of colorectal cancer among women. J Natl Cancer Inst 1996; 88: 1375-1382.

[5] Giovannucci E, Liu Y, Rimm EB, Hollis BW, Fuchs CS, Stampfer MJ and Willett WC. Pro-

spective study of predictors of vitamin D status and cancer incidence and mortality in men. J Natl Cancer Inst 2006; 98: 451-459.
[6] Pritchard RS, Baron JA and Gerhardsson de Verdier M. Dietary calcium, vitamin D, and the risk of colorectal cancer in Stockholm, Sweden. Cancer Epidemiol Biomarkers Prev 1996; 5: 897-900.
[7] Marcus PM, Newcomb PA. The association of calcium and vitamin D, and colon and rectal cancer in Wisconsin women. Int J Epidemiol 1998; 27: 788-793.
[8] Kampman E, Slattery ML, Caan B and Potter JD. Calcium, vitamin D, sunshine exposure, dairy products and colon cancer risk (United States). Cancer Causes Control 2000; 11: 459-466.
[9] Lipworth L, Rossi M, McLaughlin JK, Negri E, Talamini R, Levi F, Franceschi S and La Vecchia C. Dietary vitamin D and cancers of the oral cavity and esophagus. Ann Oncol 2009; 20: 1576-1581.
[10] Ikezaki S, Nishikawa A, Furukawa F, Tanakamura Z, Kim HC, Mori H and Takahashi M. Chemopreventive effects of 24R,25-dihydroxyvitamin D3, a vitamin D3 derivative, on glandular stomach carcinogenesis induced in rats by N-methyl-N'-nitro-N-nitrosoguanidine and sodium chloride. Cancer Res 1996; 56: 2767-2770.
[11] La Vecchia C, Ferraroni M, D'Avanzo B, Decarli A and Franceschi S. Selected micronutrient intake and the risk of gastric cancer. Cancer Epidemiol Biomarkers Prev 1994; 3: 393-398.
[12] Jourdan JL, Akhter J, Bowrey P, Pourgholami M and Morris DL. Hepatic intra-arterial injection of lipiodol-1,25-dihydroxyvitamin D3 for hepatocellular carcinoma in rats. Anticancer Res 2000; 20: 2739-2744.
[13] Pourgholami MH, Morris DL. 1,25-Dihydroxyvitamin D3 in lipiodol for the treatment of hepatocellular carcinoma: cellular, animal and clinical studies. J Steroid Biochem Mol Biol 2004; 89-90: 513-518.
[14] Pourgholami MH, Akhter J, Lu Y and Morris DL. In vitro and in vivo inhibition of liver cancer cells by 1,25-dihydroxyvitamin D3. Cancer Lett 2000; 151: 97-102.
[15] Yamamoto N, Kumashiro R. Conversion of vitamin D3 binding protein (group-specific component) to a macrophage activating factor by the stepwise action of B-galactosidase of B cells and sialidase of T cells. J Immunol 1993; 151: 2794-2802.
[16] Yamamoto N, Naraparaju VR. Role of vitamin D3-binding protein in activation of mouse macrophages. J Immunol 1996; 157: 1744-1749.
[17] Kisker O, Onizuka S, Becker CM, Fannon M, Flynn E, D'Amato R, Zetter B, Folkman J, Ray R, Swamy N and Pirie-Shepherd S. Vitamin D binding proteinmacrophage activating factor (DBP-maf) inhibits angiogenesis and tumor growth in mice. Neoplasia 2003; 5: 32-40.
[18] Yamamoto N, Suyama H, Yamamoto N and Ushijima N. Immunotherapy of metastatic breast cancer patients with vitamin D-binding protein-derived macrophage activating factor (GcMAF). Int J Cancer 2008; 122: 461-467.
[19] Sinotte M, Diorio C, Berube S, Pollak M and Brisson J. Genetic polymorphisms of the vitamin D binding protein and plasma concentrations of 25-hydroxyvitamin D in premenopausal women. Am J Clin Nutr 2009; 89: 634-640.
[20] Engelman CD, Fingerlin TE, Langefeld CD, Hicks PJ, Rich SS, Wagenknecht LE, Bowden DW and Norris JM. Genetic and environmental determinants of 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D levels in Hispanic and African Americans. J Clin Endocrinol Metab 2008; 93: 3381-3388.
[21] Ahn J, Yu K, Stolzenberg-Solomon R, Simon KC, McCullough ML, Gallicchio L, Jacobs EJ, Ascherio A, Helzlsouer K, Jacobs KB, Li Q, Weinstein SJ, Purdue M, Virtamo J, Horst R, Wheeler W, Chanock S, Hunter DJ, Hayes RB, Kraft P and Albanes D. Genome-wide association study of circulating vitamin D levels. Hum Mol Genet 2010; 19: 2739-2745.
[22] Wang TJ, Zhang F, Richards JB, Kestenbaum B, van Meurs JB, Berry D, Kiel DP, Streeten EA, Ohlsson C, Koller DL, Peltonen L, Cooper JD, O'Reilly PF, Houston DK, Glazer NL, Vandenput L, Peacock M, Shi J, Rivadeneira F, McCarthy MI, Anneli P, de Boer IH, Mangino M, Kato B, Smyth DJ, Booth SL, Jacques PF, Burke GL, Goodarzi M, Cheung CL, Wolf M, Rice K, Goltzman D, Hidiroglou N, Ladouceur M, Wareham NJ, Hocking LJ, Hart D, Arden NK, Cooper C, Malik S, Fraser WD, Hartikainen AL, Zhai G, Macdonald HM, Forouhi NG, Loos RJ, Reid DM, Hakim A, Dennison E, Liu Y, Power C, Stevens HE, Jaana L, Vasan RS, Soranzo N, Bojunga J, Psaty BM, Lorentzon M, Foroud T, Harris TB, Hofman A, Jansson JO, Cauley JA, Uitterlinden AG, Gibson Q, Järvelin MR, Karasik D, Siscovick DS, Econs MJ, Kritchevsky SB, Florez JC, Todd JA, Dupuis J, Hyppönen E and Spector TD. Common genetic determinants of vitamin D insufficiency: a genome-wide association study. Lancet 2010; 376: 180-188.
[23] McCullough ML, Stevens VL, Diver WR, Feigelson HS, Rodriguez C, Bostick RM, Thun MJ and Calle EE. Vitamin D pathway gene polymorphisms, diet, and risk of postmenopausal breast cancer: a nested case-control study. Breast Cancer Res 2007; 9: R9.
[24] Abbas S, Linseisen J, Slanger T, Kropp S, Mutschelknauss EJ, Flesch-Janys D and Chang-Claude J. The Gc2 allele of the vitamin D binding protein is associated with a decreased postmenopausal breast cancer risk, independent of the vitamin D status. Cancer Epidemiol Biomarkers Prev 2008; 17: 1339-1343.

[25] Anderson LN, Cotterchio M, Cole DE and Knight JA. 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] Kidd LC, Paltoo DN, Wang S, Chen W, Akereyeni F, Isaacs W, Ahaghotu C and Kittles R. Sequence variation within the 5' regulatory regions of the vitamin D binding protein and receptor genes and prostate cancer risk. Prostate 2005; 64: 272-282.

[27] Poynter JN, Jacobs ET, Figueiredo JC, Lee WH, Conti DV, Campbell PT, Levine AJ, Limburg P, Le Marchand L, Cotterchio M, Newcomb PA, Potter JD, Jenkins MA, Hopper JL, Duggan DJ, Baron JA and Haile RW. Genetic variation in the vitamin D receptor (VDR) and the vitamin D-binding protein (GC) and risk for colorectal cancer: results from the Colon Cancer Family Registry. Cancer Epidemiol Biomarkers Prev 2010; 19: 525-536.

[28] Flohil SC, de Vries E, van Meurs JB, Fang Y, Stricker BH, Uitterlinden AG and Nijsten T. Vitamin D-binding protein polymorphisms are not associated with development of (multiple) basal cell carcinomas. Exp Dermatol 2010; 19: 1103-1105.

[29] Schaid DJ, Rowland CM, Tines DE, Jacobson RM and Poland GA. Score tests for association between traits and haplotypes when linkage phase is ambiguous. Am J Hum Genet 2002; 70: 425-434.

[30] Welsh J. Vitamin D and prevention of breast cancer. Acta Pharmacol Sin 2007; 28: 1373-1382.

[31] Braun A, Bichlmaier R and Cleve H. Molecular analysis of the gene for the human vitamin-D-binding protein (group-specific component): allelic differences of the common genetic GC types. Hum Genet 1992; 89: 401-406.

[32] Arnaud J, Constans J. Affinity differences for vitamin D metabolites associated with the genetic isoforms of the human serum carrier protein (DBP). Hum Genet 1993; 92: 183-188.

[33] Lauridsen AL, Vestergaard P and Nexo E. Mean serum concentration of vitamin D-binding protein (Gc globulin) is related to the Gc phenotype in women. Clin Chem 2001; 47: 753-756.

[34] Lauridsen AL, Vestergaard P, Hermann AP, Brot C, Heickendorff L, Mosekilde L and Nexo E. Plasma concentrations of 25-hydroxy-vitamin D and 1,25-dihydroxy-vitamin D are related to the phenotype of Gc (vitamin D-binding protein): a cross-sectional study on 595 early postmenopausal women. Calcif Tissue Int 2005; 77: 15-22.