RESEARCH ARTICLE

Lack of Associations between Vitamin D Metabolism-Related Gene Variants and Risk of Colorectal Cancer

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Abstract

Purpose: With regard to the protective effect of vitamin D against colorectal cancer (CRC), we evaluated genetic variants that might influence vitamin D metabolism: vitamin D receptor (VDR), vitamin D binding protein (GC), vitamin D 25-hydroxylase (CYP2R1), and vitamin D 25-hydroxy 1-alpha hydroxylase (CYP27B1). Materials and Methods: A total of 657 subjects, including 303 cases with CRC and 354 controls were enrolled in this case-control study. All 657 were genotyped for the four gene variants using PCR-RFLP methods. Results: In this study, no significant difference was observed for VDR (rs2238136), GC (rs4588), CYP2R1 (rs12794714), and CYP27B1 (rs3782130) gene variants in either genotype or allele frequencies between the cases with CRC and the controls and this lack of difference remained even after adjustment for age, BMI, sex, smoking status, NSAID use, and family history of CRC. Furthermore, no evidence for effect modification of the variants and CRC by BMI, sex, or tumor site was observed. Conclusions: Our findings do not support a role for VDR, GC, and CYP27B1 genes in CRC risk in our Iranian population. Another interesting finding, which to our knowledge has not been reported previously, was the lack of association with the CYP2R1 gene polymorphism. Nonetheless, our findings require confirmation and possible roles of vitamin D metabolism-related genes in carcinogenesis need to be further investigated.

Keywords: Colorectal cancer - CYP27B1 - CYP2R1 - GC - Variant - VDR - risk - lack of association

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Introduction

Colorectal cancer (CRC) is a major health problem and is the second leading cause of cancer-related mortality (Jemal et al., 2004). Previous studies have demonstrated that high calcium intake may lower the risk of colon cancer or CRC (Kampman et al., 2000; Huncharek et al., 2009). Accumulating evidence demonstrates that vitamin D has anti-carcinogenic effects in the large bowel, and negative associations between CRC and serum levels of 25-hydroxyvitamin D [25(OH) D] (Woolcott et al., 2010) have been found. Furthermore, vitamin D reduces expression of multiple antiapoptotic proteins and promotes induction of apoptosis (Guzey et al., 2002). Vitamin D/vitamin D receptor (VDR) complex inhibits the activity of β-catenin (Palmer et al., 2001; Egan et al., 2010) and 1, 25-dihydroxyvitamin D [1, 25(OH)2 D] has a possible role in β-catenin/APC cross talk (Egan et al., 2010). Vitamin D 25-hydroxylase (CYP2R1) is the primary enzyme responsible for the conversion of vitamin D to 25-hydroxyvitamin D [25(OH) D], which is further metabolized to 1, 25(OH)2 D by the action of vitamin D 25-hydroxy 1-alpha hydroxylase (CYP27B1). Recently, significant associations between CYP2R1 gene polymorphisms and circulating levels of 25(OH) D have been found (Bu at al., 2010). Group-specific component (GC, also known as vitamin D binding protein) is another key protein of vitamin D metabolism pathway that could bind to and transport 25(OH) D to target tissues. GC gene variants have also been shown to alter plasma levels of 25(OH) D (Sinotte et al., 2009; Hibler et al., 2012).

The relationship between vitamin D metabolism-related gene variants and CRC risk has been performed to investigate their implication with CRC, but the results are somewhat contradictory for VDR (Flugge et al., 2007; Hughes et al., 2011; Mahmoudi et al., 2011; Mahmoudi et al., 2012) and GC (Poynter et al., 2010; Hibler et al., 2012; Zhou et al., 2012). Furthermore,

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Table 1. Information for the Studied Markers in the VDR, GC, CYP2R1, and CYP27B1 Genes

Gene (SNP ID)	Location (Base change)	Forward Primer Reverse Primer	PCR program (35 cycles)	PCR fragment size (bp)	Restriction enzyme Incubation temperature	Alleles: RFLP fragments size (bp)
VDR	5'-UTR	5'-CAGCATGCCTGTCCTCAGC-3'	93ºC 45s,68ºC	135	Bpu10I, 37ºC	Allele A: 135
(rs2238136)	(G/A)	5'-CCAGTACTGCCAGCTCCC-3'	25s, 72ºC 40s			Allele G: 72+63
GC	Exon 11	5'- ACATGTAGTAAGACCTTA-3'	93ºC 45s, 54ºC	233	StyI, 37ºC	Allele C: 233
(rs4588)	(A/C)	5'- GATTGGAGTGCATACGTT-3'	30s, 72ºC 45s			Allele A: 151+82
CYP2R1	Exon 1	5'-GGAAGCTTTGGAGAGCTGAA-3'	93ºC 45s, 55ºC	303	FokI, 55ºC	Allele A: 303
(rs12794714)	(A/G)	5'-GCCATAAGTCCAACCAGGAA-3'	25s, 72ºC 45s			Allele G: 173+130
CYP27B1	Promoter	5'-GTGTTCCCTAAGTGTTGTCTC-3'	93ºC 45s, 64ºC	666	TaqI, 65ºC	Allele C: 666
(rs3782130)	(C/G)	5'-GCTGACTCGGTCTCCTCTG-3'	20s, 72ºC 35s			Allele G: 367+299

only one study has examined the association between CYP27B1 gene polymprphism and CRC risk, which did not find any association (Dong et al., 2009). In addition, to our knowledge, no studies to date have evaluated the association between CYP2R1 gene variants and CRC risk.

Accordingly, these observations led we look for the possible associations of the VDR (rs2238136), GC (rs4588), CYP2R1 (rs12794714), and CYP27B1 (rs3782130) gene variants with CRC risk. Selection criteria for these polymorphisms were based on (a) their use in previous genetic epidemiology studies (b) degree of heterozygosity (c) position in the gene (d), and functional importance.

Materials and Methods

Participants

The study population consisted of 303 cases with CRC (age range, 21-87 years) and 354 controls (age range, 18-83 years) reporting to Gastroenterology and Liver Diseases Research Center, Shahid Beheshti University of Medical Sciences. This study was conducted as a case-control study and all the 657 subjects were recruited from patients who were under going colonoscopy either because of various gastrointestinal symptoms or because of they were considered high risk for CRC. Cases were the patient with positive pathologic report for CRC, and eligibility criteria for control subjects included no individual history of colorectal malignancy or polyps (including adenomatous and other polyps). Both patients and healthy controls were enrolled in the study between May 2009 and February 2012 and all of them were Iranian and genetically unrelated. Before subject's colonoscopy, self administration had been used to collect the information about their demographic, anthropometric, and clinical characteristics. The present study was approved by the Ethical Committee of Gastroenterology and Liver Diseases Research Center and all study participants were informed about the aims of the study and gave written consents. Body mass index (BMI) of each subject was calculated as body weight divided by height squared (kg/m^2) and the 657 subjects were divided in the subgroups based on the diagnosis of CRC and BMI values as follows: normal weight (BMI<25kg/m^2) controls (n=180); overweight/obese (BMI≥25kg/m^2) controls (n=174); normal weight cases with CRC (n=146); and overweight/obese cases with CRC (n=157).

Genotype analysis

Blood samples from all 657 subjects, including 303 cases with CRC and 354 controls were collected in tubes containing EDTA as an anticoagulant and store at 4ºC. Genomic DNA was purified from peripheral blood leucocytes using standard "phenol chloroform" method. In this study, genotyping was done by using PCR-RFLP method. Also, genotyping process was performed by investigators who were blinded to the participants' clinical data. Details all of the four studied polymorphisms, PCR conditions, and RFLP conditions are presented in Table 1. The PCR products were digested overnight with the appropriate restriction enzymes (Fermentas, Leon-Rot, Germany) and the digested products were run on 2 to 3% agarose gels and stained with ethidium bromide for visualization under UV light. The concordance of genotyping was confirmed by duplicate analysis of approximately 10% of the samples and DNA sequencing of approximately 3% of the samples that all of them were selected randomly (all results were accurate).

Table 2. Selected Characteristics of the Study Subjects[a]

Variables	Controls (n=354)	Cases (n=303)	p-value
Age (years)	43.9(16.7)	55.7 (12.5)	<0.001
BMI(kg/m^2)	25.0 (4.0)	25.4 (4.7)	0.216
Gender			
Men	181 (51.1)	165 (54.5)	
Women	173 (48.9)	138 (45.5)	0.395
Smoking history			
No	294 (83.0)	247 (81.5)	
Former	48 (13.6)	37 (12.2)	
Current	12 (3.4)	19 (6.3)	0.207
Regular NSAID use			
No	283 (79.9)	289 (95.4)	
Yes	71 (20.1)	14 (4.6)	<0.001
Family history of colorectal cancer			
No	308 (87.0)	264 (87.1)	
Yes	46 (13.0)	39 (12.9)	0.963
Tumor site			
Colon	-	195 (64.4)	
Rectal	-	108 (35.6)	-
Metastasis			
No	-	276 (91.1)	
Yes	-	27 (8.9)	-
HNPCC			
No	-	282 (93.1)	
Yes	-	21 (6.9)	-

[a]Variables presented as mean (SD) or number

Statistical methods

We calculated differences in demographic or anthropometric factors using t-test or χ^2 test when appropriate. Testing Hardy-Weinberg equilibrium for each of the four gene variants among cases and controls,

separately, and comparisons of the distribution of the allele frequencies between the groups were performed using the χ^2 test. Comparisons of the distribution of the genotype frequencies between the different groups were performed using the logistic regression. Logistic regression analysis was also used to adjust for confounders such as age, BMI, sex, smoking status, NSAID use, and family history of CRC. The odds ratios (OR) are given with the respective 95% confidence intervals (95%CI). SPSS statistical package (version 15.0; SPSS Inc. Chicago, IL, USA) was used to analyze the data. In all statistical tests, a p-value of less than 0.05 was considered to indicate statistical significance.

Results

Selected characteristics of the study population and their statistical significance are summarized in Table 2. On average, cases with CRC were older (p<0.001) and less likely to use NSAIDs (p<0.001) when compared with their control counterparts. However, there were no significant differences between the cases with CRC and the controls in terms of BMI, sex, smoking status, and family history of colorectal cancer.

The distribution of genotypes and alleles of the VDR (rs2238136), GC (rs4588), CYP2R1 (rs12794714), and CYP27B1 (rs3782130) gene variants in cases with CRC and controls are provided in Table 3. None of the genotype frequency distributions for these four gene variants deviated significantly from the Hardy-Weinberg equilibrium in both cases and controls, suggesting that the alleles are in equilibrium (p>0.05). Furthermore, as shown in Table 3, after adjustment for confounding factors such as age, BMI, sex, smoking status, NSAID use, and family history of CRC, no significant difference was observed in genotype and allele frequencies between the cases with CRC and the controls for the rs2238136, rs4588, rs12794714, and rs3782130 polymorphisms.

Additionally, when we stratified the analyses by sex, BMI, or tumor site (data not shown), we found no significant differences in the VDR, GC, CYP2R1, and CYP27B1 gene variants either before or after adjustment for confounding factors. Finally, in this study the risk of obesity in relation to the genes was also examined (data not shown). We observed no significant difference in genotype and allele frequencies between the normal weight cases with CRC and overweight/obese cases with CRC and between normal weight controls and overweight/obese controls for these four gene variants.

Table 3. The Genotype and Allele Frequencies of VDR, GC, CYP2R1, and CYP27B1 Gene Variants in Cases with Colorectal Cancer and Controls[a]

Gene (Variant)	Controls (n=354)	Cases (n=303)	OR (95% CI) pvalue [b]
VDR (rs2238136)			
Genotype-wise comparison			
GG	191 (54.0)	160 (52.8)	1.0(reference)
GA	137 (38.7)	109 (36.0)	1.09(0.76-1.55)0.644
AA	26 (7.3)	34 (11.2)	1.74(0.96-3.15)0.067
GA and AA	163 (46.0)	143 (47.2)	1.20(0.86-1.67)0.295
AA versus others	26 (7.3)	34 (11.2)	1.68(0.95-2.99)0.076
Allele-wise comparison			
G	519 (73.3)	429 (70.8)	1.0(reference)
A	189 (26.7)	177 (29.2)	1.13(0.89-1.43)0.311
GC (rs4588)			
Genotype-wise comparison			
CC	202 (57.1)	202 (57.1)	1.0(reference)
CA	132 (37.3)	132 (37.3)	0.94(0.66-1.33)0.724
AA	20 (5.6)	20 (5.6)	1.07(0.53-2.18)0.852
CA and AA	152 (42.9)	152 (42.9)	0.96(0.68-1.34)0.794
AA versus others	20 (5.6)	20 (5.6)	1.10(0.55-2.20)0.796
Allele-wise comparison			
C	536 (75.7)	536 (75.7)	1.0(reference)
A	172 (24.3)	172 (24.3)	0.98(0.76-1.26)0.877
CYP2R1 (rs12794714)			
Genotype-wise comparison			
GG	110 (31.1)	93 (32.1)	1.0(reference)
GA	167 (47.2)	135 (46.6)	1.04(0.70-1.52)0.866
AA	77 (21.7)	62 (21.4)	0.96(0.61-1.53)0.875
GA and AA	244 (69.0)	197 (68.0)	1.01(0.71-1.45)0.957
AA versus others	77 (21.7)	62 (21.4)	0.95(0.63-1.42)0.783
Allele-wise comparison			
G	387 (54.7)	321 (55.3)	1.0(reference)
A	321 (45.3)	259 (44.3)	0.97(0.78-1.21)0.806
CYP27B1 (rs3782130)			
Genotype-wise comparison			
GG	180 (50.8)	144 (47.5)	1.0(reference)
GC	138 (39.0)	125 (41.3)	1.15(0.81-1.63)0.448
CC	36 (10.2)	34 (11.2)	1.28(0.73-2.23)0.386
GC and CC	174 (49.2)	159 (52.5)	1.17(0.84-1.64)0.345
CC versus others	36 (10.2)	34 (11.2)	1.20(0.71-2.05)0.498
Allele-wise comparison			
G	498 (70.3)	413 (68.2)	1.0(reference)
C	210 (29.7)	193 (31.8)	1.12(0.88-1.40)0.391

[a]Variables presented as number (%); [b]Adjusted for age, BMI, sex, smoking status, NSAID use, and family history in genotype-wise comparisons

Discussion

Currently, CRC is considered as a complex disease that might result from the interaction between genetic and environmental factors. The number and nature of genes that influence susceptibility to CRC are largely unknown. However, the protective effect of calcium in CRC is well established and is the subject of recent interest. Furthermore, some of the vitamin D metabolism-related gene polymorphisms such as VDR variants appear to be involved in maintaining calcium homeostasis (Howard et al., 1995). In the present investigation, we conducted a case-control study to explore the possible association between the VDR (rs2238136), GC (rs4588), CYP2R1 (rs12794714), and CYP27B1 (rs3782130) gene variants and CRC risk among Iranians. In this study, no statistically significant difference was found for these variants in either genotype or allele frequencies between the cases and controls. Furthermore, no evidence for effect modification of the association these four genes and CRC by BMI, sex, or tumor site was observed. Accordingly, our findings do not support a role for effect of the vitamin D metabolism-related gene variants investigated and CRC risk.

Vitamin D is demonstrated to regulate cell proliferation and differentiation (Bureau et al., 1997; Stein et al., 1997). Also, VDR is involved in the Wnt/β-catenin signaling pathway that is central to colon cancer. The influence of the most VDR gene polymorphisms on VDR protein function is largely unknown to date. The -4817 G>A (rs2238136) variant is located in intron 1 at the 5'-untranslated region (5'-UTR) of VDR gene and considering that 5'-UTR

polymorphisms were found to influence the expression of VDR gene (Fang et al., 2005), we hypothesized that -4817 G>A variant might be associated with susceptibility or resistance to CRC. The association between VDR genetic polymorphisms and CRC have been examined in several epidemiologic studies, and the results were contradictory (Flugge et al., 2007; Hughes et al., 2011; Mahmoudi et al., 2011; Mahmoudi et al., 2012). Only three studies have evaluated the association between VDR gene -4817 G>A variant and CRC risk up to now (Flugge et al., 2007; Hughes et al., 2011; Mahmoudi et al., 2012). In accord with the results obtained by Flugge et al. (2007) and Hughes et al. (2011) we did not detect any significant difference in the distribution of VDR -4817 G>A genotypes or alleles between cases with CRC and the controls. On the other hand, in a study of Mahmoudi et al. (2012) the "AA" genotype of VDR gene -4817 G>A variant appeared to be a marker of increased CRC susceptibility.

The other gene studied here, GC, is involved in transport and binding of vitamin D metabolites. Our results are in line with recent studies by Hibler et al. (2012) and by Poynter et al. (2010), where no association was found between the GC rs4588 (Thr 420 Lys) variant and CRC risk; nevertheless, significant association has been reported (Zhou et al., 2012). Hibler et al. (2012) showed significant associations between other GC polymorphisms and circulating levels of 25(OH) D. It has been suggested that the increased risk of subjects carrying the GC 420Lys/Lys variant to develop CRC (Zhou et al., 2012) may be due to their lower serum 25(OH) D as compared with individuals in the GC Thr/Thr variant (Sinotte et al., 2009).

Inconsistent results such as those described for VDR and GC gene polymorphisms are common in genetic association studies (Ioannidis et al., 2001). Discrepancy in these studies may be due to false positive results, differences in the genetic and/or environmental factors triggering the development of CRC, variation in dietary intakes including calcium, small sample size, and statistical methods. Alternatively, the VDR rs2238136 and GC rs4588 variants may be in linkage disequilibrium with other unknown functional variants of the respective genes that explains the discrepancy observed.

CYP27B1 and CYP2R1 are the two other vitamin D metabolism-related genes which their associations with CRC risk were investigated in this study. Previous studies have shown the presence of CYP27B1 in the colon endothelium (Zehnder et al., 2001) and only one study has examined the association between CYP27B1 gene polymprphism and CRC risk (Dong et al., 2009). In accord with the results obtained by Dong et al. (2009) our study do not provide evidence for a major effect of the CYP27B1 polymorphism on CRC risk. Furthermore, our study represents the first investigation into the association of CYP2R1 gene polymorphism with CRC risk; no significant association was found for the gene in the overall analysis and in the analyses stratified by BMI, sex, or tumor site. Since rs12794714 is a synonymous polymorphism of Ser to Ser in exon 1 meaning that it does not alter the amino acid sequence of CYP2R1, the exact molecular mechanism responsible for the possible biological effects of the variation is not known at present. However, to conclude that there is no relationship between the CYP2R1 rs12794714 variant and CRC risk, it should be further studied in other populations.

Although well-designed, this study has several limitations. One limitation is that only one variant of each of the four genes was genotyped and thus coverage of the gene was incomplete. The other limitation is colonoscopy-based study, and the population may not be representative of the general population. Accordingly, we could not completely rule out the possibility of chance findings. Nevertheless, the possibility of true finding should not be excluded.

In conclusion, these data do not support a role for effect of VDR, GC, and CYP27B1 genes on CRC risk in Iranian population. Furthermore, to our knowledge, this study represents the first investigation into the association of CYP2R1 gene polymorphism with CRC risk; no significant association was also found for the gene. However, although in the present study the vitamin D metabolism-related gene variants did not independently alter risk of CRC, it is possible that these genes could modify the risk in the context of serum levels of vitamin D and calcium. Accordingly, our findings require confirmation and the role of vitamin D metabolism-related genes in carcinogenesis needs to be further investigated.

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References

Bu FX, Armas L, Lappe J, et al (2010). Comprehensive association analysis of nine candidate genes with serum 25-hydroxy vitamin D levels among healthy Caucasian subjects. *Hum Genet*, **128**, 549-56.

Bureau JP, Henry L, Baz A, Scherrer K, Chateau MT (1997). Prosomes (proteasomes) changes during differentiation are related to the type of inducer. *Mol Biol Rep*, **24**, 57-62.

Dong LM, Ulrich CM, Hsu L, et al (2009). Vitamin D related genes, CYP24A1 and CYP27B1, and colon cancer risk. *Cancer Epidemiol Biomarkers Prev*, **18**, 2540-8.

Egan JB, Thompson PA, Vitanov MV, et al (2010). Vitamin D receptor ligands, adenomatous polyposis coli, and the vitamin D receptor FokI polymorphism collectively modulate beta-catenin activity in colon cancer cells. *Mol Carcinog*, **49**, 337-52.

Fang Y, van Meurs JB, d'Alesio A, et al (2005). Promoter and 3'-untranslated-region haplotypes in the vitamin d receptor gene predispose to osteoporotic fracture: the rotterdam study. *Am J Hum Genet*, **77**, 807-23.

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.

Guzey M, Kitada S, Reed JC (2002). Apoptosis induction by 1α, 25-Dihydroxyvitamin D3 in prostate cancer. *Mol Cancer Ther*, **1**, 667-77.

Hibler EA, Hu C, Jurutka PW, Martinez ME, Jacobs ET (2012). Polymorphic variation in the GC and CASR genes and

associations with vitamin D metabolite concentration and metachronous colorectal neoplasia. *Cancer Epidemiol Biomarkers Prev*, **21**, 368-75.

Howard G, Nguyen T, Morrison N, et al (1995). Genetic influences on bone density: physiological correlates of vitamin D receptor gene alleles in premenopausal women. *J Clin Endocrinol Metab*, **80**, 2800-5.

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.

Huncharek M, Muscat J, Kupelnick B (2009). Colorectal cancer risk and dietary intake of calcium, vitamin D, and dairy products: a meta-analysis of 26,335 cases from 60 observational studies. *Nutr Cancer*, **61**, 47-69.

Ioannidis JP, Ntzani EE, Trikalinos TA, Contopoulos-Ioannidis DG (2001). Replication validity of genetic association studies. *Nat Genet*, **29**, 306-9.

Jemal A, Tiwari RC, Murray T, et al (2004). American Cancer Society (2004) Cancer statistics. *CA Cancer J Clin*, **54**, 8-29.

Kampman E, Slattery ML, Caan B, Potter JD (2000). Calcium, vitamin D, sunshine exposure, dairy products and colon cancer risk (United States). *Cancer Causes Control*, **11**, 459-466.

Mahmoudi T, Arkani M, Karimi K, et al (2012). The-4817 G>A (rs2238136) variant of the vitamin D receptor gene: a probable risk factor for colorectal cancer. *Mol Biol Rep*, **39**, 5277-82.

Mahmoudi T, Karimi K, Mohebbi SR, Fatemi SR, Zali MR (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.

Palmer HG, Gonzalez-Sancho JM, Espada J, et al. (2001). Vitamin D3 promotes the differentiation of colon carcinoma cells by the induction of E-cadherin and the inhibition of β-catenin signaling. *J Cell Biol*, **154**, 369-87.

Poynter JN, Jacobs ET, Figueiredo JC, et al (2010). 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*, **19**, 525-36.

Sinotte M, Diorio C, Bérubé S, Pollak M, Brisson J (2009). Genetic polymorphisms of the vitamin D binding protein and plasma concentrations of 25-hydroxyvitamin D in premenopausal women. *Am J Clin Nutr*, **89**, 634-40.

Stein GS, Lian JB, Van Wijnen AJ, Stein JL (1997). The osteocalcin gene: a model for multiple parameters of skeletal-specific transcriptional control. *Mol Biol Rep*, **24**, 185-96.

Woolcott CG, Wilkens LR, Nomura AM, et al (2010). Plasma 25-hydroxyvitamin D levels and the risk of colorectal cancer: The multiethnic cohort study. *Cancer Epidemiol Biomarkers Prev*, **19**, 130-4.

Zehnder D, Bland R, Williams MC, et al (2001). External expression of 25-hydroxyvitamin D(3)-1alpha hydroxylase. *J Clin Endocrinol Metab*, **86**, 888-94.

Zhou L, Zhang X, Chen X, et al (2012). GC Glu416Asp and Thr420Lys polymorphisms contribute to gastrointestinal cancer susceptibility in a Chinese population. *Int J Clin Exp Med*, **5**, 72-9.