Polymorphisms of VDR gene and risk of gastric cardiac adenocarcinoma in Chinese population

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ABSTRACT

Vitamin D receptor (VDR) gene polymorphisms have been reported to increase susceptibility to some malignant tumors, yet the effect on gastric cardiac adenocarcinoma susceptibility remains unknown. Here, we conducted a hospital-based case-control study to examine the correlation of single nucleotide polymorphisms of VDR rs2107301T>C, rs2228570C>T, rs1989969C>T and rs11568820 G>A and gastric cardiac adenocarcinoma susceptibility. A total 330 cases and 608 controls were enrolled in the study. Using ligation detection reaction, we found that the variant alleles of the four polymorphisms were not associated with risk of gastric cardiac adenocarcinoma. Further stratified analyses showed that there was an increased risk associated with VDR rs1989969 polymorphism among patients who were drinking or aged <60. The haplotypes VDR T rs2107301T rs2228570C rs1989969G rs11568820T reduced the susceptibility. This study demonstrated that VDR rs1989969 polymorphism was involved in the carcinogenesis of gastric cardiac adenocarcinoma, especially increased the risk in the younger and alcohol drinking Chinese population.

INTRODUCTION

Gastric cardia adenocarcinoma (GCA) remains one of the most common malignant tumor worldwide[1]. Although the incidence of noncardia gastric cancer has declined steadily, the incidence and mortality of GCA are continuously increasing[2]. The etiology of GCA comprises interactions of multiple environmental and genetic factors. Environmental factors including cigarette smoking and alcohol consumption[3, 4], as well as genetic factors such as gene polymorphisms, have been implicated with GCA. Yet, the underlying etiological mechanisms of GCA are not fully understood.

The 1,25-dihydroxyvitamin D3 [1,25(OH)2D3] is the hormonally active form of vitamin D, which has been shown to inhibit prostate, breast and colon cancer cell progression[5]. Consistently, amounting evidence has indicated the correlation of the Vitamin D Receptor (VDR) and cancer. The antineoplastic effect of 1,25(OH)2D3 requires the expression of VDR in tumor cells[6]. Increased tumor VDR expression is associated with a better prognosis in various types of cancer[7, 8]. The association of single nucleotide polymorphisms (SNPs) in VDR (ApaI [rs7975232], BsmI [rs1544410], FokI [rs10735810], TaqI [rs731236] and cancer risk has been reported, yet the results were inconclusive [6, 9–12].
In our previous study, we have investigated the correlation of four SNPs \textit{VDR} rs11568820 G>A, \textit{VDR} rs1989969 C>T, \textit{VDR} rs2107301 T>C and \textit{VDR} rs2228570 C>T with esophageal squamous cell carcinoma development, and found that \textit{VDR} rs2107301 T>C polymorphism with alcohol drinking enhanced the risk of esophageal squamous cell carcinoma[13]. Considering GCA occurs in the immediate vicinity of esophagus, we hypothesized that these four SNPs are also related to GCA. Here, we performed a hospital-based case-control study to examine the genetic effects of these four SNPs on the development of GCA.

### RESULTS

#### Characteristics of the study subjects

The characteristics of the study subjects, including demographics and environmental risk factors, are presented in Table 1. The cases and controls were well matched in gender and age ($\chi^2$ test, $p=0.746$ and 0.965, respectively). However, tobacco smoking rate was much higher in GCA patients as compared with the control subjects (36.67% vs. 27.96%, $p=0.006$). Alcohol drinking rate was higher, yet not significantly, in GCA patients than in control subjects (29.39% vs. 23.01%, $p=0.072$).

As shown in Table 2, the genotyping successful rates were ranging from 95.76% to 100.0% in GCA cases and from 95.39 to 99.18% in controls. Compared with the minor allele frequency (MAF) for Chinese in database for all four SNPs loci, the MAF in our controls was similar (Table 2). In the control subjects, the genotype frequencies for these four polymorphisms reached Hardy-Weinberg equilibrium ($p$-value for HWE, all $p>0.05$, Table 2).

#### Associations between risk of GCA and four polymorphisms

As demonstrated in Table 3, the single locus analyses showed no statistically significant difference in genotype frequencies of four SNPs between the cases and the controls ($p>0.05$). There are no correlation between these four polymorphic sites with the risk of GCA as evaluated by the logistic regression analyses (Table 3).

#### Stratification analyses of four polymorphisms and risk of GCA

To further assess the effects of \textit{VDR} rs11568820 G>A, rs1989969 C>T, rs2107301 T>C and rs2228570 C>T on GCA risk according to different sex, smoking, age and alcohol drinking status, stratification analyses were conducted as shown in Tables 4–7, respectively. In association with the \textit{VDR} rs1989969 C>T polymorphism, we further identified two significantly increased risk factors of GCA, which are age<60 or alcohol drinking.

#### Linkage disequilibrium analyses

Linkage disequilibrium analyses in both controls and cases were conducted as shown in Table 8. $D^\prime$ and
were analyzed and showed that there were weak correlations between the four loci.

Haplotype analysis of VDR polymorphisms and susceptibility of GCA

As shown in Table 9, haplotype analysis was also conducted and haplotypes were from the genotypes of VDR polymorphisms. The haplotype analysis indicated that the VDR T(2107301)T-C(2228570)T-C(1989969)T-G(11568820) was the most common haplotype in both groups (17.27% in case group, 14.88% in control group). Compared with the haplotype T(2107301)T-C(2228570)T-C(1989969)T-G(11568820) the haplotypes VDR T(2107301)T-C(2228570)T-C(1989969)G(11568820) were more common in the controls (0.143) than in the case group (0.113) with significant difference ($p=0.038$). T(2107301)T-C(2228570)T-C(1989969)G(11568820) haplotype was associated with a significantly reduced risk of GCA (OR=0.68, 95%CI=0.48-0.98, $p=0.038$). We also further conducted other haplotypes and risk of GCA, but no association was observed between patients and controls.

DISCUSSION

In the current study, by multivariable logistic analysis, we demonstrated that there was no significant association between the polymorphisms of VDR rs11568820 G>A, rs1989969 C>T, rs2107301 T>C and rs2228570 C>T and the risk of GCA in Chinese population. Yet, notably, we detected an increased risk of GCA among alcohol drinking or younger patients (<60 of age) who carried VDR rs1989969 C>T genotype. Interestingly, the VDR T(2107301)T-C(2228570)T-C(1989969)G(11568820) haplotype was associated with a significantly reduced risk of GCA.

Recently, accumulating evidence showed 1,25(OH)$_2$D$_3$, the hormonally active form of vitamin D, participates in apoptosis, cell proliferation and inflammation in cancer[5]. 1,25(OH)$_2$D$_3$ could restrain cancer cell growth by inducing their differentiation, by arresting cells in the $G_0$/$G_1$ phase of cell cycle or by induction of apoptotic cell death. Additionally, 1,25(OH)$_2$D$_3$ also has an impact on angiogenesis, thereby limiting the invasiveness of cancer cells[6]. As the key component of the vitamin D metabolism process, VDR similarly participates in the regulation of cancer development.

Table 2: Primary information for VDR rs2107301 T>C, rs2228570 C>T, rs1989969 C>T and rs11568820 G>A polymorphisms

| Genotyped SNPs | VDR rs2107301 T>C | VDR rs2228570 C>T | VDR rs1989969 C>T | VDR rs11568820 G>A |
|---------------|-------------------|-------------------|-------------------|-------------------|
| Chromosome    | 12                | 12                | 12                | 12                |
| Gene (ID)     | VDR (7421)        | VDR (7421)        | VDR (7421)        | VDR (7421)        |
| Function      | Intron region     | Missense          | Intron region     | Intergene region  |
| Chr Pos (Genome Build 36.3) | 46541837        | 46559162          | 46564277          | 46588812          |
| Regulome DB Score$^a$ | 5             | 5                 | No data           | No data           |
| TFBS$^b$      | —                 | —                 | —                 | Y                |
| Splicing (ESE or ESS) | —              | Y                 | —                 | —                |
| nsSNP         | —                 | Y                 | —                 | —                |
| MAF$^c$ for Chinese in database | 0.291          | 0.482             | 0.330             | 0.453             |
| MAF in our controls (n = 608) | 0.297          | 0.456             | 0.323             | 0.433             |
| $p$ value for HWE$^d$ test in our controls | 0.690          | 0.347             | 0.718             | 0.574             |
| Genotyping method$^e$ | LDR            | LDR               | LDR               | LDR              |
| % Genotyping value | 95.52%         | 95.52%            | 98.19%            | 98.08%            |

$^a$ http://www.regulomedb.org/;

$^b$ TFBS: Transcription Factor Binding Site (http://snpinfo.niehs.nih.gov/snpinfo/snpfunc.htm);

$^c$ MAF: minor allele frequency;

$^d$ HWE: Hardy–Weinberg equilibrium;

$^e$ LDR: ligation detection reaction.
Table 3: Logistic regression analyses of associations between VDR rs2107301 T>C, rs2228570 C>T, rs1989969 C>T and rs11568820 G>A polymorphisms and risk of GCA

| Genotype                  | Cases (n = 330) | Controls (n = 608) | Crude OR (95%CI) | p   | Adjusted OR (95%CI) | p   |
|---------------------------|-----------------|--------------------|-------------------|-----|---------------------|-----|
| **VDR rs2107301 T>C**     |                 |                    |                   |     |                     |     |
| TT                        | 155 49.05       | 285 49.14          | 1.00              |     | 1.00                |     |
| TC                        | 129 40.82       | 246 42.41          | 0.96 (0.72-1.29)  | 0.819 |
| CC                        | 32  10.13       | 49  8.45           | 1.20 (0.74-1.95)  | 0.492 |
| **CC vs. TC vs. TT**      |                 |                    |                   |     |                     |     |
| TC+CC                     | 161 50.95       | 295 50.86          | 1.00 (0.76-1.32)  | 0.982 |
| TT+TC                     | 284 89.87       | 531 91.55          | 1.00              |     |                     |     |
| CC                        | 32  10.13       | 49  8.45           | 1.22 (0.77-1.95)  | 0.436 |
| **C allele**              | 193 58.54       | 344 29.66          |                   |     |                     |     |
| **VDR rs2228570 C>T**     |                 |                    |                   |     |                     |     |
| CC                        | 97  30.70       | 166 28.62          | 1.00              |     | 1.00                |     |
| CT                        | 153 48.42       | 299 51.55          | 0.88 (0.64-1.20)  | 0.488 |
| TT                        | 66  20.89       | 115 19.83          | 0.98 (0.66-1.46)  | 0.895 |
| **TT vs. CT vs. CC**      |                 |                    |                   |     |                     |     |
| CT+TT                     | 219 69.30       | 414 71.38          | 0.91 (0.67-1.22)  | 0.666 |
| CC+CT                     | 250 79.11       | 465 80.17          | 1.00              |     |                     |     |
| TT                        | 66  20.89       | 115 19.83          | 1.07 (0.76-1.50)  | 0.799 |
| **T allele**              | 285 45.09       | 529 45.60          |                   |     |                     |     |
| **VDR rs1989969 C>T**     |                 |                    |                   |     |                     |     |
| CC                        | 135 42.45       | 278 46.10          | 1.00              |     | 1.00                |     |
| CT                        | 140 44.03       | 260 43.12          | 1.11 (0.83-1.48)  | 0.521 |
| TT                        | 43  13.52       | 65 10.78           | 1.36 (0.88-2.11)  | 0.172 |
| **TT vs. CT vs. CC**      |                 |                    |                   |     |                     |     |
| CT+TT                     | 183 57.55       | 325 53.90          | 1.16 (0.88-1.53)  | 0.314 |
| CC+CT                     | 275 86.48       | 538 89.22          | 1.00              |     |                     |     |
| TT                        | 43  13.52       | 65 10.78           | 1.29 (0.86-1.95)  | 0.221 |
| **T allele**              | 226 35.53       | 390 32.34          |                   |     |                     |     |

(Continued)
frequently associated with carcinogenesis are FokI (rs2228570), BsmI (rs1544410), TaqI (rs731236), ApaI (rs7975232) and Cdx2 (rs11568820)[10, 19, 20], yet the results are inconsistent. In contrast to the findings that VDR gene polymorphisms seem not related to the esophageal adenocarcinoma (EAC) risk development[21], we previously showed a significantly increased risk of esophageal squamous cell carcinoma associated with VDR rs2107301 T>C polymorphism among patients who were drinking[13]. Therefore, in this study, we sought to verify our hypothesis that SNPs in VDR gene is related to GCA since it occurs in the anatomical vicinity of esophagus. Similarly, none of the four polymorphic sites was associated with the change of susceptibility to GCA, but a remarkable increased risk of GCA was found among alcohol drinking but younger patients (<60 years of age) who carried VDR rs1989969 C>T genotype.

Previous studies have shown the correlation of several SNPs with GCA (summarized in[22]). PLCE1 (rs2274223) A>G SNP causes a missense variation in the protein phospholipase-Cε-1, which generates two critical messengers [inositol 1,4,5-triphosphate (IP3) and diacylglycerol (DAG)], thereby affecting cell growth, differentiation and gene expression[23]. Interestingly, vitamin D [(1,25(OH)2D3] – VDR signaling pathway also stimulates the IP3 and DAG generation via phospholipase C[24], which may probably underlie its correlation with GCA. Other genetic variants significantly correlated with the risk of GCA included PRKAA1 (rs13361707), IL1B (rs16944), TNF (rs1800629) and MDM2 (rs2279744)[22], involving the signal transduction, inflammation, apoptosis aspects. However, the connections between these SNPs with our findings remain obscure.

As compared with the homozygote for the common allele, men who were homozygote for the rare allele for VDR rs2107301 have higher risk of prostate cancer[25]. In contrast, VDR rs2107301 was not associated with GCA in either single locus analyses or the stratified analyses in the current study. Instead, we demonstrated that VDR rs1989969 C>T polymorphism increased the risk of GCA among younger patients or alcohol drinkers, exemplifying the significance of the environment and genetic risk factors interact and both contribute to the carcinogenesis.

Our study showed the VDR T rs2107301 T>rs2228570 C G haplotype was associated with a significantly reduced risk of GCA, which indicated that polymorphism in single locus might not significantly modify the risk of cancer. The chain effect lying in different loci leads to a more profound impact which could regulate the risk of cancer.

Between ethnic groups, the frequencies of genetic polymorphisms do vary. In our study, the allele frequency of VDR rs1989969 was 0.323 in 608 control subjects, which is consistent with that in the Chinese Han (0.330) in the SNP Database, but lower than that of African (0.510) and Caucasian (0.410) population (http://www.ncbi.nlm.nih.gov/SNP).

Considering VDR rs1989969 C>T mutant alleles in the control group, ORs, GCA samples and control samples, the power of our analysis (α = 0.05) was 0.999 in 330 GCA cases and 608 controls with an OR of 2.05 in age<60 subgroup, and 0.983 with an OR of 1.78 in the drinking subgroup (PS, version 3.0, 2009, available

| Genotype | Cases (n = 330) | Controls (n = 608) | Crude OR (95%CI) | p | Adjusted OR (95%CI) | p |
|----------|----------------|-------------------|-----------------|---|-------------------|---|
|          | n   | %  | n   | %  |                |    |                  |    |
| VDR rs11568820 G>A |          |      |      |      |                |    |                  |    |
| GG       | 99  | 30.00 | 193 | 32.71 | 1.00 | 1.00 | 0.500 |
| GA       | 162 | 49.09 | 283 | 47.97 | 1.12 | 0.488 | (0.82-1.52) | 0.500 |
| AA       | 69  | 20.91 | 114 | 19.32 | 1.18 | 0.399 | (0.80-1.73) | 0.361 |
| AA vs. GA vs. GG |          |      |      |      | 0.666 |
| GA+AA    | 231 | 70.00 | 397 | 67.29 | 1.13 | 0.397 | (0.85-1.52) | 0.390 |
| GG+GA    | 261 | 79.09 | 476 | 80.68 | 1.00 | 1.00 | 0.503 |
| AA       | 69  | 20.91 | 114 | 19.32 | 1.10 | 0.563 | (0.79-1.54) | 0.503 |
| A allele | 300 | 45.45 | 511 | 43.31 |      |      |      |      |

* Adjusted for age, sex, smoking status and alcohol consumption.
| Variable | VDR rs1989969 C>T (case/control) | Adjusted OR* (95% CI); p; \( p_h \)^c | TT vs. (CT+CC) |
|----------|---------------------------------|-----------------------------------|-----------------|
|          | CC     | CT     | TT     | CT+TT | 1.00 | CC     | CT     | TT     | CT+TT | 1.00 |
| Sex      |        |        |        |        |      |        |        |        |        |      |
| Male     | 92/189 | 98/170 | 28/46  | 126/216 | 1.00 | 1.19 (0.83-1.69); \( p_h \): 0.345; \( p_h \): 0.523 | 1.26 (0.74-2.14); \( p_h \): 0.405; \( p_h \): 0.575 | 1.20 (0.86-1.68); \( p_h \): 0.283; \( p_h \): 0.734 | 1.15 (0.70-1.91); \( p_h \): 0.579; \( p_h \): 0.412 |
|          | 1.00   |        |        |        |      | 1.00   |        |        |        |      |
| Female   | 43/89  | 42/90  | 15/19  | 57/109 | 1.00 | 1.00 (0.59-1.70); \( p_h \): 0.999; \( p_h \): 0.523 | 1.67 (0.76-3.66); \( p_h \): 0.199; \( p_h \): 0.575 | 1.12 (0.68-1.84); \( p_h \): 0.660; \( p_h \): 0.734 | 1.67 (0.80-3.50); \( p_h \): 0.172; \( p_h \): 0.412 |
| Age      |        |        |        |        |      |        |        |        |        |      |
| <60      | 31/90  | 42/65  | 15/14  | 57/79  | 1.00 | 1.00 (1.03-3.33); \( p_h \): 0.041; \( p_h \): 0.031 | 2.97 (1.24-7.13); \( p_h \): 0.015; \( p_h \): 0.010 | 2.05 (1.18-3.57); \( p_h \): 0.006; \( p_h \): 0.096 | 2.19 (0.97-4.97); \( p_h \): 0.006; \( p_h \): 0.096 |
| ≥60      | 104/188| 98/195 | 28/51  | 126/246| 1.00 | 0.90 (0.64-1.27); \( p_h \): 0.560; \( p_h \): 0.063 | 0.99 (0.59-1.67); \( p_h \): 0.977; \( p_h \): 0.043 | 0.92 (0.67-1.27); \( p_h \): 0.621; \( p_h \): 0.010 | 0.90 (0.64-1.71); \( p_h \): 0.865; \( p_h \): 0.006 |
| Smoking status |      |        |        |        |      |        |        |        |        |      |
| Never    | 88/197 | 87/189 | 25/48  | 112/237| 1.00 | 1.01 (0.70-1.46); \( p_h \): 0.962; \( p_h \): 0.482 | 1.12 (0.64-1.97); \( p_h \): 0.696; \( p_h \): 0.345 | 1.03 (0.73-1.46); \( p_h \): 0.863; \( p_h \): 0.359 | 1.11 (0.65-1.90); \( p_h \): 0.692; \( p_h \): 0.451 |
| Ever     | 47/81  | 53/71  | 18/17  | 71/88  | 1.00 | 1.24 (0.74-2.10); \( p_h \): 0.415; \( p_h \): 0.482 | 1.90 (0.87-4.15); \( p_h \): 0.107; \( p_h \): 0.345 | 1.37 (0.84-2.24); \( p_h \): 0.213; \( p_h \): 0.359 | 1.71 (0.82-3.56); \( p_h \): 0.154; \( p_h \): 0.451 |
| Alcohol consumption |      |        |        |        |      |        |        |        |        |      |
| Never    | 99/205 | 93/206 | 30/47  | 123/253| 1.00 | 0.90 (0.63-1.27); \( p_h \): 0.538; \( p_h \): 0.057 | 1.31 (0.77-2.23); \( p_h \): 0.315; \( p_h \): 0.835 | 0.97 (0.70-1.35); \( p_h \): 0.865; \( p_h \): 0.100 | 1.39 (0.84-2.28); \( p_h \): 0.202; \( p_h \): 0.647 |
| Ever     | 36/73  | 47/54  | 13/18  | 60/72  | 1.00 | 1.90 (1.04-3.46); \( p_h \): 0.036; \( p_h \): 0.057 | 1.45 (0.60-3.50); \( p_h \): 0.409; \( p_h \): 0.835 | 1.78 (1.01-3.14); \( p_h \): 0.045; \( p_h \): 0.100 | 1.05 (0.46-2.40); \( p_h \): 0.903; \( p_h \): 0.647 |

*a The genotyping was successful in 318 (96.36%) GCA cases and 603 (99.18%) controls for VDR rs1989969 C>T;  
b Adjusted for age, sex, smoking status and alcohol consumption (besides stratified factors accordingly) in a logistic regression model;  
c \( p_h \) for heterogeneity; bold values are statistically significant (\( p < 0.05 \)).
Table 5: Stratified analyses between VDR rs2228570 polymorphism and GCA risk by sex, age, smoking status and alcohol consumption

| Variable     | VDR rs2228570 (case/control)* | Adjusted OR (95% CI); p; p\textsubscript{h} | Adjusted OR (95% CI); p; p\textsubscript{h} |
|--------------|--------------------------------|--------------------------------------------|--------------------------------------------|
|              | CT    | CC    | TT    | CT+TT | TT    | CT    | CC    | CT+CC | CC vs. (CT+TT) |
| Sex          |       |       |       |       |       |       |       |       |                |
| Male         | 101/205 | 71/113 | 40/73 | 141/278 | 1.112 | (0.71-1.71); p: 0.646; p\textsubscript{h}: 0.572 | 0.894 | (0.49-1.62); p: 0.711; p\textsubscript{h}: 0.572 | 0.807 | (0.56-1.16); p: 0.242; p\textsubscript{h}: 0.984 |
| Female       | 52/94 | 26/53 | 26/42 | 78/136 | 1.00 | 1.112 | (0.54-1.42); p: 0.581; p\textsubscript{h}: 0.559 | 0.894 | (0.40-1.56); p: 0.501; p\textsubscript{h}: 0.559 | 0.807 | (0.66-1.55); p: 0.953; p\textsubscript{h}: 0.965 |
| Age          |       |       |       |       |       |       |       |       |                |
| <60          | 45/78 | 25/48 | 15/33 | 60/111 | 1.00 | 1.269 | (0.62-2.58); p: 0.511; p\textsubscript{h}: 0.945 | 0.786 | (0.52-1.19); p: 0.258; p\textsubscript{h}: 0.945 | 0.786 | (0.52-1.19); p: 0.511; p\textsubscript{h}: 0.945 |
| ≥60          | 108/221 | 72/118 | 51/82 | 159/303 | 1.00 | 1.146 | (0.53-2.49); p: 0.732; p\textsubscript{h}: 0.745 | 0.981 | (0.62-1.55); p: 0.935; p\textsubscript{h}: 0.745 | 0.981 | (0.62-1.55); p: 0.935; p\textsubscript{h}: 0.745 |
| Smoking status |       |       |       |       |       |       |       |       |                |
| Never        | 108/221 | 69/117 | 45/78 | 153/299 | 1.00 | 0.847 | (0.55-1.31); p: 0.452; p\textsubscript{h}: 0.062 | 1.016 | (0.53-1.95); p: 0.961; p\textsubscript{h}: 0.062 | 0.847 | (0.55-1.31); p: 0.452; p\textsubscript{h}: 0.062 |
| Ever         | 45/78 | 28/49 | 21/37 | 66/115 | 1.00 | 1.022 | (0.64-1.64); p: 0.927; p\textsubscript{h}: 0.722 | 1.007 | (0.49-2.04); p: 0.985; p\textsubscript{h}: 0.722 | 1.022 | (0.64-1.64); p: 0.927; p\textsubscript{h}: 0.722 |
| Alcohol consumption |       |       |       |       |       |       |       |       |                |
| Never        | 122/228 | 72/125 | 50/86 | 172/314 | 1.00 | 0.920 | (0.61-1.39); p: 0.693; p\textsubscript{h}: 0.051 | 0.916 | (0.53-1.66); p: 0.536; p\textsubscript{h}: 0.051 | 0.920 | (0.61-1.39); p: 0.693; p\textsubscript{h}: 0.051 |
| Ever         | 31/71 | 25/41 | 16/29 | 47/100 | 1.00 | 0.991 | (0.63-1.56); p: 0.968; p\textsubscript{h}: 0.893 | 1.105 | (0.50-2.43); p: 0.803; p\textsubscript{h}: 0.893 | 0.991 | (0.63-1.56); p: 0.968; p\textsubscript{h}: 0.893 |

* The genotyping was successful in 316 (95.75%) GCA cases and 603 (95.70%) controls VDR rs2228570 C>T;
* Adjusted for age, sex, smoking status and alcohol consumption (besides stratified factors accordingly) in a logistic regression model;
* p\textsubscript{h} for heterogeneity; bold values are statistically significant (p <0.05).
| Variable          | VDR rs2107301 (case/control) | Adjusted OR (95% CI); p; p<sup>h</sup>  |
|-------------------|-------------------------------|------------------------------------------|
|                   | CC       | CT   | TT       | CT+TT  | CC       | CT   | TT       | CT+TT  | TT vs. (CT+CC) |
| **Sex**           |          |      |          |        |          |      |          |        |                |
| Male              | 21/30    | 86/174 | 105/187  | 191/361| 1.00     | 0.706 | (0.38-1.31); | 0.802  | (0.43-1.47);   | 0.756  | (0.42-1.36);   | 0.934  | (0.669-1.31); |
|                   |          |      |          |        |          |      |          |        |                |
|                   |          |      |          |        |          |      |          |        |                |
| Female            | 11/19    | 43/72  | 50/98    | 93/170 | 1.00     | 1.032 | (0.45-2.37); | 0.881  | (0.39-1.99);   | 0.945  | 1.163           |
|                   |          |      |          |        |          |      |          |        |                |
| **Age**           |          |      |          |        |          |      |          |        |                |
| <60               | 9/15     | 36/71  | 40/73    | 76/144 | 1.00     | 0.845 | (0.34-2.12); | 0.913  | (0.37-2.27);   | 0.880  | (0.37-2.1);    | 0.955  | (0.56-1.62);   |
|                   |          |      |          |        |          |      |          |        |                |
| ≥60               | 23/34    | 93/175 | 115/212  | 208/387| 1.00     | 0.786 | (0.44-1.41); | 0.802  | (0.45-1.43);   | 0.795  | 1.023           |
|                   |          |      |          |        |          |      |          |        |                |
| **Smoking status**|          |      |          |        |          |      |          |        |                |
| Never             | 24/36    | 94/176 | 104/204  | 198/380| 1.00     | 0.801 | (0.45-1.42); | 0.765  | (0.43-1.35);   | 1.279  | (0.74-2.21);   | 1.092  | (0.78-1.51);   |
|                   |          |      |          |        |          |      |          |        |                |
| Ever              | 8/13     | 35/70  | 51/81    | 86/151 | 1.00     | 0.813 | (0.31-2.14); | 1.023  | (0.39-2.64);   | 0.925  | 0.823           |
|                   |          |      |          |        |          |      |          |        |                |
| **Alcohol consumption** |          |      |          |        |          |      |          |        |                |
| Never             | 25/42    | 103/189| 116/208  | 219/397| 1.00     | 0.916 | (0.53-1.58); | 0.937  | (0.54-1.62);   | 0.927  | (0.55-1.56);   | 0.994  | (0.73-1.36);   |
|                   |          |      |          |        |          |      |          |        |                |
| Ever              | 7/7      | 26/57  | 39/77    | 65/134 | 1.00     | 0.456 | (0.15-1.43); | 0.506  | (0.16-1.55);   | 0.485  | (0.16-1.44);   | 1.018  | (0.57-1.80);   |

*a The genotyping was successful in 316 (95.75%) GCA cases and 603 (95.39%) controls for VDR rs1989969 C>T;*  
*b Adjusted for age, sex, smoking status and alcohol consumption (besides stratified factors accordingly) in a logistic regression model;*  
*c p<sub>h</sub> for heterogeneity; bold values are statistically significant (p <0.05).*
| Variable     | VDR rs11568820 (case/control) | Adjusted OR (95% CI); p; ρ<sub>h</sub> | GG vs. (AG+AA) |
|--------------|-------------------------------|----------------------------------------|----------------|
|              | AA | AG | GG | AG+GG | AA | AG | GG | AG+GG |                      |
| Sex          |     |    |    |       | 49/85 100/184 74/126 174/310 | 0.943 (0.65-1.45); ρ<sub>a</sub>: 0.787; ρ<sub>b</sub>: 0.399 | 0.974 (0.65-1.45); ρ<sub>a</sub>: 0.927; ρ<sub>b</sub>: 0.895 |
| Male         |     |    |    |       | 1.00 | 1.019 (0.65-1.60); ρ<sub>a</sub>: 0.936; ρ<sub>b</sub>: 0.352 | 0.943 (0.66-1.34); ρ<sub>a</sub>: 0.743; ρ<sub>b</sub>: 0.895 |
| Female       | 20/29 62/99 25/67 87/166 | 1.00 | 0.908 (0.47-1.74); ρ<sub>a</sub>: 0.772; ρ<sub>b</sub>: 0.399 | 0.943 (0.66-1.34); ρ<sub>a</sub>: 0.743; ρ<sub>b</sub>: 0.895 |
| Age          |     |    |    |       | <60 | 1.689 (0.82-3.48); ρ<sub>a</sub>: 0.153; ρ<sub>b</sub>: 0.799 | 0.775 (0.51-1.23); ρ<sub>a</sub>: 0.224; ρ<sub>b</sub>: 0.799 |
| <60          | 14/34 48/69 27/60 75/129 | 1.00 | 1.093 (0.51-2.36); ρ<sub>a</sub>: 0.821; ρ<sub>b</sub>: 0.151 | 0.780 (0.53-1.15); ρ<sub>a</sub>: 0.207; ρ<sub>b</sub>: 0.824 |
| ≥60          | 55/80 114/214 72/133 186/347 | 1.00 | 1.412 (0.71-2.79); ρ<sub>a</sub>: 0.824; ρ<sub>b</sub>: 0.844 | 1.062 (0.75-1.49); ρ<sub>a</sub>: 0.732; ρ<sub>b</sub>: 0.844 |
| Smoking status |     |    |    |       | Never | 0.958 (0.63-1.45); ρ<sub>a</sub>: 0.839; ρ<sub>b</sub>: 0.214 | 0.799 (0.51-1.32); ρ<sub>a</sub>: 0.338; ρ<sub>b</sub>: 0.151 |
| Never        | 50/83 120/208 65/135 185/343 | 1.00 | 0.895 (0.60-1.33); ρ<sub>a</sub>: 0.582; ρ<sub>b</sub>: 0.200 | 1.213 (0.85-1.72); ρ<sub>a</sub>: 0.280; ρ<sub>b</sub>: 0.191 |
| Ever         | 19/31 42/75 34/58 76/133 | 1.00 | 0.914 (0.46-1.81); ρ<sub>a</sub>: 0.796; ρ<sub>b</sub>: 0.214 | 0.932 (0.49-1.76); ρ<sub>a</sub>: 0.902; ρ<sub>b</sub>: 0.200 |
| Alcohol consumption |     |    |    |       | Never | 0.829 (0.56-1.24); ρ<sub>a</sub>: 0.359; ρ<sub>b</sub>: 0.176 | 0.790 (0.54-1.15); ρ<sub>a</sub>: 0.218; ρ<sub>b</sub>: 0.150 |
| Never        | 58/84 126/220 73/145 199/365 | 1.00 | 1.202 (0.58-1.66); ρ<sub>a</sub>: 0.282; ρ<sub>b</sub>: 0.145 | 1.084 (0.86-1.68); ρ<sub>a</sub>: 0.282; ρ<sub>b</sub>: 0.145 |
| Ever         | 11/30 36/63 26/48 62/111 | 1.00 | 1.558 (0.69-3.48); ρ<sub>a</sub>: 0.277; ρ<sub>b</sub>: 0.176 | 1.523 (0.52-1.69); ρ<sub>a</sub>: 0.274; ρ<sub>b</sub>: 0.150 |

a The genotyping was successful in 330 (100%) GCA cases and 603 (97.04%) controls for VDR rs11568820 A>G;

b Adjusted for age, sex, smoking status and alcohol consumption (besides stratified factors accordingly) in a logistic regression model;

c ρ<sub>h</sub> for heterogeneity; bold values are statistically significant (p <0.05).
We acknowledge that there are several limitations in the current study: First, restrained by the moderate sample size and lack of a validation cohort, the statistical power of our study was limited. Larger studies in multiple ethnic populations and various geographic locations are demanded to confirm the associations reported in our study. Second, the genetic effects of $VDR$ polymorphisms on GCA susceptibility are probably caused by linkage disequilibrium (LD) with several functional variations within the $VDR$ gene or with other closely linked genes. The SNPs we chose to study may not serve as a comprehensive representative of all the genetic variability at http://biostat.mc.vanderbilt.edu/twiki/bin/view/Main/PowerSampleSize).

Table 8: Linkage disequilibrium analyses of $VDR$ rs2228570, rs1989969, rs11568820 and rs2107301 in control and case groups

|                | Control       | Case          |
|----------------|---------------|---------------|
|                | rs2228570     | rs1989969     | rs11568820    | rs2228570 | rs1989969 | rs11568820 |
| $D'$           | 0.289         | 0.224         | 0.136         | 0.202     | 0.105     | 0.015      |
| rs2107301      | -             | 0.213         | 0.069         | -         | 0.206     | 0.138      |
| rs1989969      | -             | -             | 0.249         | -         | -         | 0.366      |
| $r^2$          | 0.029         | 0.010         | 0.006         | 0.015     | 0.003     | 0.000      |

Table 9: $VDR$ haplotype frequencies (%) in cases and controls and risk of GCA

| Haplotypes | Cases (n = 660) | Controls (n = 1216) | Crude OR (95% CI) | $p$ |
|------------|-----------------|---------------------|-------------------|-----|
|            | $n$             | %                   | $n$               | %   |       |       |
| $VDR$ $T$  | $rs2107301$ $C$ | $rs2228570$ $C$     | $rs1989969$ $G$  | $rs11568820$ | 114  | 17.27 | 181  | 14.88 | 1.00  |
| $VDR$ $T$  | $rs2107301$ $T$ | $rs2228570$ $C$     | $rs1989969$ $G$  | $rs11568820$ | 75   | 11.36 | 174  | 14.31 | 0.68 (0.48-0.98) | 0.038 |
| $VDR$ $T$  | $rs2107301$ $C$ | $rs2228570$ $C$     | $rs1989969$ $A$  | $rs11568820$ | 54   | 8.18  | 123  | 10.12 | 0.70 (0.47-1.04) | 0.075 |
| $VDR$ $T$  | $rs2107301$ $T$ | $rs2228570$ $T$     | $rs1989969$ $A$  | $rs11568820$ | 74   | 11.21 | 114  | 9.38  | 1.03 (0.71-1.50) | 0.875 |
| $VDR$ $C$  | $rs2107301$ $C$ | $rs2228570$ $C$     | $rs1989969$ $G$  | $rs11568820$ | 59   | 8.94  | 115  | 9.46  | 0.82 (0.55-1.21) | 0.305 |
| $VDR$ $T$  | $rs2107301$ $T$ | $rs2228570$ $C$     | $rs1989969$ $A$  | $rs11568820$ | 44   | 6.67  | 86   | 7.07  | 0.81 (0.53-1.25) | 0.346 |
| $VDR$ $C$  | $rs2107301$ $C$ | $rs2228570$ $C$     | $rs1989969$ $A$  | $rs11568820$ | 31   | 4.70  | 65   | 5.35  | 0.76 (0.47-1.23) | 0.264 |
| $VDR$ $T$  | $rs2107301$ $T$ | $rs2228570$ $T$     | $rs1989969$ $G$  | $rs11568820$ | 29   | 4.39  | 63   | 5.18  | 0.73 (0.44-1.20) | 0.218 |
| $VDR$ $T$  | $rs2107301$ $C$ | $rs2228570$ $C$     | $rs1989969$ $A$  | $rs11568820$ | 36   | 5.45  | 62   | 5.10  | 0.92 (0.58-1.48) | 0.736 |
| $VDR$ $T$  | $rs2107301$ $T$ | $rs2228570$ $T$     | $rs1989969$ $G$  | $rs11568820$ | 34   | 5.15  | 58   | 4.77  | 0.93 (0.57-1.51) | 0.771 |
| $VDR$ $C$  | $rs2107301$ $T$ | $rs2228570$ $C$     | $rs1989969$ $A$  | $rs11568820$ | 27   | 4.09  | 50   | 4.11  | 0.86 (0.51-1.45) | 0.565 |
| $VDR$ $C$  | $rs2107301$ $C$ | $rs2228570$ $T$     | $rs1989969$ $G$  | $rs11568820$ | 15   | 2.27  | 31   | 2.55  | 0.77 (0.40-1.49) | 0.433 |
| $VDR$ $C$  | $rs2107301$ $T$ | $rs2228570$ $T$     | $rs1989969$ $A$  | $rs11568820$ | 20   | 3.03  | 29   | 2.38  | 1.10 (0.59-2.03) | 0.773 |
| $VDR$ $C$  | $rs2107301$ $T$ | $rs2228570$ $G$     | $rs1989969$ $G$  | $rs11568820$ | 17   | 2.58  | 25   | 2.06  | 1.08 (0.56-2.09) | 0.820 |
| $VDR$ $C$  | $rs2107301$ $C$ | $rs2228570$ $G$     | $rs1989969$ $G$  | $rs11568820$ | 24   | 3.64  | 23   | 1.89  | 1.66 (0.89-3.07) | 0.109 |
| $VDR$ $C$  | $rs2107301$ $T$ | $rs2228570$ $G$     | $rs1989969$ $A$  | $rs11568820$ | 7    | 1.06  | 17   | 1.40  | 0.65 (0.26-1.63) | 0.361 |

With the order of $VDR$ rs2107301 T>C, rs2228570 C>T, rs1989969 C>T and rs11568820 G>A in gene position.
of VDR, which entails further studies clarifying the genetic mechanism of GCA carcinogenesis by fine-mapping the susceptible region of the variants. Third, the study subjects recruited were from hospitals in the east part of China with same ethnicity, which may compromise its representativeness of the general population for potential inherited bias. Last but no least, the biological effects of VDR rs1989969 C>T polymorphism on VDR function and the downstream signaling cascade remain unclear. Located on the second intron of VDR, rs1989969 may probably cause an alternative RNA splicing on VDR mRNA, thereby regulating the VDR protein function. Yet this speculation demands further investigations.

In conclusion, the GCA is associated with a variety of factors including gene, environment and life-style. Our findings that the increased risk of GCA was found among alcohol drinking and younger patients (<60 years of age) who carried VDR rs1989969 C>T genotype and the reduced risk of GCA for man with VDR T rs2107301 T rs2228570 C>T rs1989969 G rs11568820 haplotype, should be interpreted with much caution. Further larger studies in multiple ethnical populations and various geographic locations are needed to verify our preliminary results.

MATERIALS AND METHODS

Ethical approval of the study protocol

This hospital-based case-control study was approved by the Review Board of Jiangsu University (Zhenjiang, China). We have complied with the World Medical Association Declaration of Helsinki regarding ethical conduct of research involving human subjects and/or animals. All subjects provided written informed consent to be included. Each participant agreed to donate 2ml of peripheral venous blood for the research project, which was performed by skilled nurses under strict aseptic condition to minimize potential risks on subjects’ well being.

Study subjects

The study included a total of 938 subjects. 330 patients with GCA were consecutively recruited from the Affiliated People’s Hospital of Jiangsu University and Affiliated Hospital of Jiangsu University (Zhenjiang, China) between October 2010 and December 2012. The exclusion criteria were patients who previously had cancer, any metastasized cancer, radiotherapy or chemotherapy. The 608 controls were patients without cancer frequency-matched to the cases with regard to age (±5 years) and sex recruited from the two hospitals mentioned above during the same time period. Most of the controls were admitted to the hospitals for the treatment of trauma. Gender and age distribution had no significant difference between the case group and the control group, respectively.

Experienced and well-trained personnel interviewed each study subject with a pretested questionnaire. Demographic data and related risk factors were collected. 2mL samples of venous blood were collected from each subject with consent. Individuals who smoked one cigarette per day for ≥1 year were defined as “smokers”. Subjects who consumed ≥3 alcoholic drinks a week for ≥6 months were considered to be “alcohol drinkers”.

Isolation of DNA and genotyping by ligation detection reaction

Blood samples from patients and controls were collected using vacuum blood tube with Ethylene Diamine Tetraacetic Acid (EDTA). Genomic DNA was isolated from whole blood by using QIAamp DNA Blood Mini Kit (Qiagen, Berlin, Germany). Gene polymorphisms were analyzed by the ligation detection reaction (LDR) method with technical support from the Biowing Applied Biotechnology (Shanghai, China). 10% of the total samples were randomly selected to repeated analyses in order to maximize the probably error of the genotyping results and improve quality control.

Statistical analyses

Statistical analyses were performed using SPSS17.0 Statistical Package (2007, SPSS Inc., Chicago, IL). Hardy-Weinberg equilibrium for genotypes was tested by goodness-of-fit χ² in control group. The distribution of VDR rs2107301 T>C, rs2228570 C>T, rs1989969 C>T and rs11568820 G>A genotypes was performed using the chi-square (χ²) test to examine statistical differences between patients and controls. The associations between these four SNPs and risk of GCA were estimated by computing the Odds ratios and confidence intervals (95%) using logistic regression analyses. Crude ORs and adjusted ORs when adjusting for age, sex, smoking and drinking status were also computed by using logistic regression analyses. Bilateral probability tests were taken, p value <0.05 on behalf of the difference was statistically significant.

Abbreviations

VDR: vitamin D receptor, GCA: gastric cardiac adenocarcinoma, LD: linkage disequilibrium, OR: odds ratio, CI: confidential interval, SNPs: single-nucleotide polymorphisms.
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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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