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Common genetic variants related to vitamin D status are not associated with esophageal squamous cell carcinoma risk in China

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Abstract

Background—Few studies have examined the association of common genetic variants related to vitamin D metabolism and signaling to esophageal squamous cell carcinoma(ESCC).

Methods—We evaluated the association between 12 single nucleotide polymorphisms(SNPs) in four genes related to vitamin D levels and ESCC risk using data from a genome-wide association study. Participants were recruited from the Shanxi Upper Gastrointestinal Cancer Genetics Project and the Linxian Nutrition Intervention Trials, and included 1942 ESCC cases and 2111 controls.

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Conflicts of interest statement

The authors have no conflicts of Interest to disclose.

We used logistic models to estimate odds ratios(ORs) and 95% confidence intervals(CIs) for the SNP associations, after controlling for age and gender.

Results—None of the 12 evaluated SNPs in the four vitamin D-related genes were significantly associated with risk of ESCC. The strongest associations were for rs3794060($P=0.07$) and rs12800438($P=0.08$) in the *DHCR7/NADSYN1* gene. No association between vitamin D-related SNPs and risk of ESCC was observed in a genotype score analysis that included all 12 SNPs. ORs for quartiles 2, 3 and 4 of the genotype scores were 0.83 (95% CI: 0.68, 1.01), 1.02(0.85, 1.21), and 1.08 (0.89, 1.30), respectively, with no evidence for a significant monotonic trend($P=0.120$).

Conclusions—Our results suggested that common genetic variants related to vitamin D levels are not associated with risk of ESCC in this Chinese population.

Keywords

vitamin D; genetic variants; esophageal squamous cell carcinoma; China

1. Introduction

Common genetic variants related to serum vitamin D levels have been identified by two genome-wide association studies (GWAS) [1, 2]. These vitamin D pathway genes include vitamin D binding protein (*GC*), 7-dehydrocholesterol reductase /NAD synthetase 1 (*DHCR7/NADSYN1*), 25-hydroxylase (*CYP2R1*), and 24-hydroxylase (*CYP24A1*). Previous epidemiologic studies that evaluated the association between serum vitamin D concentrations and risk of esophageal squamous cell carcinoma (ESCC) either showed elevated risk in men [3] or no effect overall [4] for higher serum 25(OH) vitamin D. Studies with other methods have also been reported [5], but no clear role for vitamin D in the etiology of ESCC has been observed. No previous studies have evaluated the association between polymorphisms in vitamin D pathway genes and risk of ESCC, which should provide strong evidence for an association because these SNPs are significantly correlated with serum vitamin D levels and may, therefore, work as instrumental variables that mark life time differences in the exposure to vitamin D [6]. In the current study we assessed this association using data from a genome-wide association study (GWAS) conducted among 4053 Chinese adults from Shanxi and Henan Provinces.

2. Methods

2.1 Study population

The study participants were drawn from two large studies, the Shanxi Upper Gastrointestinal Cancer Genetics Project (Shanxi) and the Linxian Nutrition Intervention Trials (NIT). A total of 4053 individuals were included in this study, including 1942 ESCC cases and 2111 controls. The participants have been described elsewhere [7].

2.2 Vitamin D related single nucleotide polymorphism (SNP)

Genome-wide scanning was performed on 4053 samples using the Illumina 660W Quad chip as described previously [7]. In this study, four vitamin D-related genes and 12 SNPs were selected based on a documented association with serum concentrations of 25-hydroxy

vitamin D in two previously published genome wide association studies [1, 2]. In these studies, SNPs in *GC*, *DHCR7/NADSYN1*, *CYP2R1* and *CYP24A1* were shown to be directly associated with circulating 25-hydroxy vitamin D concentration. We therefore used these SNPs as surrogate measures of lifetime vitamin D exposure in the manner of a Mendelian randomization study [6] (**Table 1**).

2.3 Statistical analysis

We tested each SNP in controls to ensure that it was in Hardy-Weinberg equilibrium. We estimated odds ratios (ORs) and 95% confidence intervals (CIs) for the association between the SNPs and ESCC risk using unconditional logistic regression in additive models controlling for age and gender. We also conducted a genotype score analysis in which risk alleles were defined as alleles associated with reduced serum 25(OH) vitamin D concentrations. For SNPs in the genes *GC*, *DHCR7/NADSYN1*, *CYP2R1* and *CYP24A1*, each participant received a score of 0, 1, or 2 for carrying 2, 1, or 0 risk alleles, respectively. The participant's overall genotype score was calculated by summing the individual risk scores, with a total possible score of zero to 24. We did not account for linkage disequilibrium in this score, which may tend to overestimate the effect. Logistic regression was used to calculate ORs and 95% CIs of the quartiles of the genotype scores, using the lowest quartile as the reference. All tests were two-sided, and $P < 0.05$ was considered statistically significant. P values were not adjusted for multiple tests. All statistical analyses were conducted using SAS software (version 9.2, SAS Institute Inc., Cary, North Carolina).

3. Results

The mean age for both cases and controls was 56 years. The cases included 778 women and 1164 men, and the controls included 677 women and 1434 men. Cases were more likely to be older, male and non-smoking (all $P < 0.001$); there was no difference in cases and controls for alcohol drinking ($P = 0.109$).

None of the 12 evaluated SNPs was associated with risk of ESCC. Of the 12 SNPs, odds ratios (ORs) were borderline non-significant for two SNPs (rs3794060 and rs12800438), both in the *DHCR7/NADSYN1* gene (**Table 2**). We also found no association between vitamin D-related SNPs and risk of ESCC using the genotype score analysis. ORs for quartiles 2 (11 to <13), 3 (13 to <16) and 4 (16) of the genotype scores were 0.83 (95%CI: 0.68, 1.01), 1.02 (0.85, 1.21), and 1.08 (0.89, 1.30), respectively, without evidence of a significant monotonic trend ($P = 0.120$) (**data not shown**).

4. Discussion

This report is the first study to examine common genetic variants of vitamin D-related genes and risk of ESCC. Overall, we found gene polymorphisms known to be associated with serum vitamin D concentrations were not associated with risk of ESCC in this population. Although a number of studies have evaluated the associations between vitamin D-related gene polymorphisms and various cancers [5, 8], little data for genetic polymorphisms in vitamin D-related genes and ESCC is available.

Our study has several strengths, including a population-based design, large sample size, and comprehensive evaluation of all SNPs with a known relation to serum vitamin D levels. Subjects in our study obtain most of their vitamin D through sun exposure, because their diets have little vitamin D and they do not consume fortified products. Furthermore, our population has low median vitamin D status [3, 9], which may make the role of genetic variants stronger. We also adjusted for potential confounders in the multivariate model, including age and gender. One potential limitation is that we had no data on serum 25(OH) vitamin D levels. However, a previous study that included eight prospective cohorts (the Vitamin D Pooling Project), found no association between serum 25(OH) vitamin D concentrations and risk of upper gastrointestinal cancers [4].

In summary, our study provides the first evaluation of common genetic variants in vitamin D-related genes and risk of ESCC. We found no association between gene polymorphisms related to serum vitamin D levels and ESCC risk in this Chinese population.

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Research highlights

1. We evaluated the association between 12 vitamin D related-SNPs and ESCC risk using data from a GWAS.
2. None of the 12 evaluated SNPs in the four vitamin D-related genes were significantly associated with risk of ESCC.
3. Our study provides the first evaluation of common genetic variants in vitamin D-related genes and risk of ESCC.

Table 1

Single nucleotide polymorphisms of vitamin D-related genes^a that were evaluated in this study.

Nearest Gene (SNPs)	Location	Present on Illumina 660W Quad chip
GC		
rs2282679 [1, 2]	72827247	Y ^b
rs1155563 [1, 2]	72862352	Y ^b
rs7041 [1, 2]	72837198	Y ^b
rs3755967[2]	72828262	N ^c
rs17467825 [2]	72824381	N ^c
rs2298850[2]	72833131	N ^c
DHCR7/NADSYN1		
rs3794060[2]	70865327	Y ^b
rs12800438 [2]	70848651	Y ^b
rs7944926[2]	70843273	Y ^b
rs3829251 [1]	70872207	Y ^b
rs12785878 [2]	70845097	N ^c
rs4945008 [2]	70898896	N ^c
rs4944957[2]	70845683	N ^c
CYP2R1		
rs2060793 [1]	14871886	Y ^b
rs1993116[2]	14866810	Y ^b
rs12794714 [2]	14870151	Y ^b
rs10500804 [2]	14866849	Y ^b
rs10741657 [2]	14871454	N ^c
rs7116978 [2]	14838347	N ^c
CYP24A1		
rs6013897 [2]	-	N ^d

^aSNPs found to be associated with serum 25-hydroxyvitamin D concentration in previous genome-wide association analyses [1,2]

^bThe SNP was included in the chip.

^cThe SNP was not included in the chip.

^dThe referenced SNP was not included on the chip, instead we used proxy rs17217119 as a proxy ($r^2 = 1.00$).

Table 2

Per allele odds ratios and 95% confidence intervals of the selected vitamin D-related SNPs and proxy-SNPs and risk of ESCC.

SNPs	Chr	Minor allele and frequency		Nearest gene	OR	95%CI	P value
rs2282679	4	C	0.32	<i>GC</i>	0.96	0.87, 1.06	0.42
rs1155563	4	C	0.40	<i>GC</i>	1.02	0.93, 1.12	0.62
rs7041	4	G	0.26	<i>GC</i>	0.98	0.89, 1.09	0.74
rs3794060	11	C	0.48	<i>DHCR7/NADSYN1</i>	0.92	0.84, 1.01	0.07
rs12800438	11	G	0.48	<i>DHCR7/NADSYN1</i>	0.92	0.85, 1.01	0.08
rs7944926	11	A	0.47	<i>DHCR7/NADSYN1</i>	0.93	0.85, 1.02	0.11
rs3829251	11	A	0.25	<i>DHCR7/NADSYN1</i>	1.02	0.92, 1.13	0.76
rs2060793	11	A	0.39	<i>CYP2R1</i>	0.99	0.91, 1.09	0.86
rs1993116	11	T	0.39	<i>CYP2R1</i>	1.00	0.91, 1.09	0.92
rs12794714	11	A	0.36	<i>CYP2R1</i>	1.01	0.92, 1.11	0.79
rs10500804	11	G	0.36	<i>CYP2R1</i>	1.01	0.93, 1.11	0.77
rs17217119	20	G	0.16	<i>CYP24A1</i>	1.01	0.90, 1.14	0.87

OR=odds ratio; 95% CI=95% confidence interval