Association between novel PLCE1 variants identified in published esophageal cancer genome-wide association studies and risk of squamous cell carcinoma of the head and neck

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Abstract

Background: Phospholipase C epsilon 1 (PLCE1) (an effector of Ras) belonging to the phospholipase family plays crucial roles in carcinogenesis and progression of several cancers, including squamous cell carcinoma of the head and neck (SCCHN). A single nucleotide polymorphism (SNP, rs2274223) in PLCE1 has been identified as a novel susceptibility locus in genome-wide association studies (GWAS) of esophageal squamous cell carcinoma (ESCC) and gastric cardia adenocarcinoma (GCA) that share similar risk factors with SCCHN. Therefore, we investigated the association between potentially functional SNPs in PLCE1 and susceptibility to SCCHN.

Methods: We genotyped three potentially functional SNPs (rs2274223A/G, rs3203713A/G and rs11599672T/G) of PLCE1 in 1,098 SCCHN patients and 1,090 controls matched by age and sex in a non-Hispanic white population.

Results: Although none of three SNPs was alone significantly associated with overall risk of SCCHN, their combined effects of risk alleles (rs2274223G, rs3203713G and rs11599672G) were found to be associated with risk of SCCHN in a locus-dose effect manner ($P_{\text{trend}} = 0.046$), particularly for non-oropharyngeal tumors ($P_{\text{trend}} = 0.017$); specifically, rs2274223 was associated with a significantly increased risk (AG vs. AA: adjusted OR = 1.29, 95% CI = 1.01-1.64; AG/GG vs. AA: adjusted OR = 1.30, 95% CI = 1.03-1.64), while rs11599672 was associated with a significantly decreased risk (GG vs. TT: adjusted OR = 0.54, 95% CI = 0.34-0.86; TG/GG vs. TT: adjusted OR = 0.76, 95% CI = 0.61-0.95).

Conclusions: Our findings suggest that PLCE1 variants may have an effect on risk of SCCHN associated with tobacco and alcohol exposure, particularly for those tumors arising at non-oropharyngeal sites. These findings, although need to be validated by larger studies, are consistent with those in esophageal and gastric cancers.

Keywords: PLCE1, polymorphism, SCCHN, risk, susceptibility

Background

Squamous cell carcinoma of the head and neck (SCCHN) is the fifth most common cancer worldwide [1], which includes malignancies at the sites of the oral cavity, larynx and pharynx. It was estimated that there were 48,010 new cases and 11,260 deaths of SCCHN in the United States in 2010 [2]. Tobacco smoke and alcohol consumption are the well-recognized risk factors for SCCHN, and more recently human papillomavirus (HPV) is recognized as a major risk factor for the oropharyngeal cancer [3]. However, accumulative evidence also shows that genetic factors, including family history and polymorphisms in genes involved in multiple biological pathways, such as carcinogen metabolism, DNA repair, cell cycle regulation, apoptosis and other cellular processes, play important roles in the etiology of SCCHN [4,5]. Although an increasing number of studies on genetic risk factors for SCCHN have been published, the exact genetic basis of susceptibility to SCCHN is still not well defined.
In the past few years, the wave of genome-wide association studies (GWASs) provided a more robust tool to find novel susceptibility loci or genes for cancer susceptibility by using high-throughput genotyping technology to interrogate a large number of tagging polymorphisms in a high density across the whole genome [6]. To date, more than 50 cancer GWASs have been published, including at least 15 different malignant tumors [7], which has greatly improved our understanding of genetic basis of human cancers. However, only one recent GWAS focused on SCCHN risk and identified five variants at 4q21, 12q24 and ADH gene cluster, significantly associated with risk of upper aerodigestive tract cancers (UATC) including SCCHN [8]. It may be a very small proportion of SNPs associated with SCCHN risk because of the strict criteria for the GWAS significance level (\(P = 10^{-7}\) or \(P = 10^{-8}\)). Thus, further exploration for the genetic variants that did not reach the GWAS significance level in the development of SCCHN is needed.

In 2010, two large-scale genome-wide association studies [9,10] simultaneously reported that a new and notable low-penetrance susceptibility locus (rs2274223) located in the phospholipase C epsilon 1 gene (PLCE1) was strongly associated with risk of esophageal squamous cell carcinoma (ESCC) and gastric cardia adenocarcinoma (GCA) in Chinese population. Rs2274223 is a non-synonymous SNP located in exon 26 of the PLCE1 gene, causing the amino acid change from histidine to arginine. Additionally, one study also showed that the positive rates of the PLCE1 protein in ESCC and GCA tissues were significantly higher than that in normal ones, suggesting a biologically plausible role of PLCE1 in carcinogenesis of human cancers [9].

The PLCE1 gene resides on chromosome 10q23 and encodes phospholipase C epsilon 1 (PLCE1), which belongs to the phospholipase family that catalyzes the hydrolysis of polyphosphoinositides to generate secondary messengers, such as inositol-1,4,5 trisphosphate and diacylglycerol. Therefore, PLCE1 is involved in cell growth and differentiation [11]. Studies have reported that PLCE1 functions as an effector of Ras and plays crucial roles in carcinogenesis and progression of several cancers, including cancers of the intestine [12], skin [13], bladder [14], colorectal [15] and head and neck [16]. All these findings further support the biological plausibility that genetic variations, such as single nucleotide polymorphisms (SNPs) in PLCE1 that affect the gene expression or protein functions, may affects the risk of some cancers.

Studies have reported that SCCHN shares some similar risk factors with ESCC and GCA, such as tobacco smoke and alcohol consumption [17-19]. Thus, the exciting results in the GWAS of ESCC and GCA, and the strong biological evidence encouraged us to investigate the association between functional SNPs in PLCE1 and susceptibility to SCCHN. In the present study, we performed genotyping analyses of three potentially functional SNPs in PLCE1 (rs2274223A/G, rs3203713A/G and rs11599672T/G) in non-Hispanic whites and assessed their associations with risk of SCCHN in our ongoing hospital-based case-control study of 1,098 SCCHN cases and 1,090 cancer-free controls matched to cases on age (± 5 years) and sex.

**Methods**

**Study populations**

Participant recruitment has been described elsewhere [20,21]. In brief, newly-diagnosed SCCHN cancer patients were consecutively recruited from The University of Texas M.D. Anderson Cancer Center between October 1999 and October 2007. All cases were diagnosed with histologically confirmed SCCHN, and there were no age, sex or stage restrictions. Exclusion criteria included second SCCHN primary tumors, primary tumors of the nasopharynx or sinonasal tract, metastasized cancer from other organs, or any histopathologic diagnosis other than SCCHN. Additionally, patients who had received prior surgery (other than diagnostic biopsies), chemotherapy or radiation therapy before recruitment, and any blood transfusion during the preceding 6 months were also excluded.

Cancer-free controls were recruited from those visitors in outpatient clinics at M.D. Anderson Cancer Center, who were not genetically related to the patients or to each other, and were frequency-matched to the cases on age (±5 years), sex and ethnicity. The inclusion criterion for controls was defined as the self-reported absence of prior history of cancer. After the first survey using a short questionnaire to find out whether potential subjects were willing to participate and qualified in this study, a written informed consent was obtained, and a structured questionnaire was administered by trained interviewers to collect demographic data and environmental exposure history, such as age, sex, ethnicity, smoking and alcohol consumption. After interview, approximately 30-ml venous blood sample was collected from each participant. The response rates for cases and controls were approximately 90% and 85%, respectively. In the analysis, a total of 1,098 cases and 1,090 controls that completed the interview and donated a one-time blood sample were included. All subjects were non-Hispanic whites. This study was approved by the Institutional Review Board of M. D. Anderson.

**SNP Selection**

Besides the non-synonymous SNP (rs2274223) identified in the published GWAS, we also searched the NCBI dbSNP database (http://www.ncbi.nlm.nih.gov/ build 131) for other common [minor allele frequency (MAF) ≥ 0.05] and potentially functional SNPs located in the 5’ near gene, 5’- and 3’-untranslated regions of PLCE1 by
using a set of tools at the website SNPInfo (http://snpinfo.niehs.nih.gov/) [22]. In addition, the LD analyses were performed to optimize SNP selection. We selected additional two potentially functional SNPs in PLCE1: rs3203713, located in the miRNA binding site (3’UTR) and rs11599672, located in the transcription factor binding site (TFBS in 5’ near gene). Taken together, we included three SNPs (rs2274223A/G, rs3203713A/G and rs11599672T/G) in PLCE1 for the final genotyping.

Genotyping
We extracted genomic DNA from the buffy-coat fraction of the blood samples using the Qiagen Blood DNA Mini Kit (Qiagen Inc., Valencia, Calif) according to the manufacturer’s instruction and genotyped SNPs rs2274223 and rs3203713 using the TaqMan allelic discrimination assay on an ABI 7900 system (Applied Biosystems). Genotyping was performed without knowing the subjects’ case or control status, and four negative controls (no DNA) and duplicated commercial positive controls (TaqMan Control Genomic DNA; Applied Biosystems) included in each 384-well plate were used for quality control. The accuracy achieved 100% for the duplicates of 5% of samples. Polymerase chain reaction-restriction fragment length polymorphisms (PCR-RFLP) method was also used to identify genotypes of rs11599672, because the Taqman assay was not applicable to this SNP. The antisense primer was introduced a mismatched A to replace T at +2bp from the polymorphic site to create an SspI restriction site (sense: 5’-GGAGAGACATTCTGTTGGTTA-3’; antisense: 5’-CCTTCAAAAACCACCGCTGTAATT-3’). A 155-bp fragment containing this T/G site was amplified in the PCR mixture consisted with approximately 20 ng of genomic DNA, 2 pmol of each primer, 0.1 mM each dNTP, 1 x PCR buffer (50 mM KCl, 10 mM Tris HCl, and 0.1% Triton X-100), 1.5 mM MgCl2, and 0.5 unit of Taq polymerase. The PCR profile consisted of an initial melting step of 95°C for 5 min; 35 cycles of 95°C for 30 s, 59°C for 45 s, and 72°C for 1 min; and final extension step of 72°C for 10 min. The PCR product was then digested with the restriction enzyme SspI (New England BioLabs, Beverly, MA) overnight at 37°C and separated on 3% agarose gel. The T allele has the restriction site and produces two SspI bands of 134- and 21-bp, whereas the G allele lacks the SspI restriction site, resulting in one band of 155-bp (Figure 1). Finally, 10% of the samples were randomly selected to perform the repeated assays, and the results were 100% concordant. Direct sequencing was also conducted to confirm the genotypes of rs11599672 (Figure 2).

Statistical analysis
We evaluated differences in selected demographic variables, risk factors, and frequencies of the PLCE1 genotypes between cases and controls by using the χ² test and examined Hardy-Weinberg equilibrium by a goodness-of-fit χ² test to compare the observed genotype frequencies with the expected ones among the controls. We estimated associations between PLCE1 variants and SCCHN risk by computing odds ratios (ORs) and 95% confidence intervals (CIs) from both univariate and multivariate logistic regression analyses with adjustment for the known risk factors for SCCHN, such as age, sex, smoking status and alcohol use. Haplotype frequencies and individual haplotypes were generated by using SAS PROC HAPLOTYPE based on the observed genotypes. All statistical analyses were two sided, and P < 0.05 was considered statistically significant. All the statistical analyses were performed with Statistical Analysis System software (v.9.1 SAS Institute, Cary, NC).

Results
Characteristics of study subjects
The characteristics of cases and controls included in this study are summarized in Table 1. The mean age was 57.1 years (±11.1) for cases and 56.6 years (±11.0) for controls. There were no significant differences in the distributions of age and sex between cases and controls (P = 0.676 and 0.581, respectively), suggesting the frequency-matching on age and sex was adequate. However, 72.2% and 72.9% of the SCCHN cases were smokers and drinkers, respectively, which were significantly higher than that of the controls (50.9% and 56.6%, respectively) (P < 0.001 for the both). Of the 1,098 cases, 559 (50.9%) had tumors of the oropharynx and 539 (49.1%) had tumors arising at non-oropharynx sites including oral cavity, hypopharynx and larynx. Furthermore, 272 cases (24.8%) presented with
stage I-II and 826 cases (75.2%) presented with III-IV stage.

Overall associations between PLCE1 variants and risk of SCCHN

The genotype and allele distributions of three SNPs (rs2274223, rs3203713 and rs11599672) in cases and controls are shown in Table 2. The observed genotype frequencies for these three polymorphisms were all in Hardy-Weinberg equilibrium in the controls ($P_{\text{HWE}} = 0.98$ and 0.72, respectively). The single locus analyses revealed that genotype distributions of these three polymorphisms were not significantly different between overall cases and controls ($P = 0.554$ for rs2274223, $P = 0.860$ for rs3203713 and $P = 0.265$ for rs11599672, respectively). However, we found that the frequencies of variant rs2274223G and rs3203713G alleles (0.335 and 0.170, respectively) among the controls were slightly higher than those in the cases (0.320 and 0.162, respectively), while the frequency of the variant rs11599672G allele among the cases was slightly lower than that among the controls (0.277 vs. 0.297), suggesting rs2274223G, rs3203713G and rs11599672G alleles may be risk alleles to be considered in further analyses.

The LD analysis showed that two PLCE1 polymorphisms rs2274223 and rs3203713 were in incomplete LD in our study population ($D' = 0.99$, $r^2 = 0.40$), but these two SNPs were not in LD with rs11599672. To estimate possible joint effects and potential locus-locus interactions of PLCE1 polymorphisms on risk of SCCHN, we then examined the combined effects of these three variants by the number of putative risk alleles (i.e. rs2274223G, rs3203713G and rs11599672T). As shown in Table 2, we trichotomized the subjects into three groups with “0-1”, “2-3” and “4-6” risk alleles. Compared with the group of “0-1” risk allele, there was a locus-dose effect as the risk allele number increased ($P_{\text{trend}} = 0.046$). After adjustment for age, sex, smoking and alcohol status, the group of “2-3” risk alleles was borderline associated with risk of SCCHN (adjusted OR = 1.20, 95% CI = 0.97-1.50, adjusted $P = 0.096$) and the “4-6” risk allele group was significantly associated with risk of SCCHN (adjusted OR = 1.31, 95% CI = 1.00-1.73, adjusted $P = 0.049$). When “2-3” and “4-6” groups were combined for a larger number in the same stratum, the association between a larger number of risk alleles and risk of SCCHN remained statistically significant (adjusted OR = 1.23, 95% CI = 1.00-1.52, adjusted $P = 0.048$).

Associations between PLCE1 variants and risk of SCCHN by tumor sites

To investigate the modifying effects of PLCE1 variants on risk of SCCHN with different tumor sites, we conducted the stratification analysis by oropharyngeal and non-oropharyngeal cancers. As shown in Table 2, rs2274223 variant genotypes were associated with a significantly increased risk of SCCHN only for non-oropharyngeal sites (AG vs. AA: adjusted OR = 1.29, 95% CI = 1.01-1.64, adjusted $P = 0.042$; AG/GG vs. AA: adjusted OR = 1.30, 95% CI = 1.03-1.64, adjusted $P = 0.025$), while rs11599672 variant genotypes were associated with a significantly decreased risk of SCCHN for this group of patients (GG vs. TT: adjusted OR = 0.54, 95% CI = 0.34-0.86, adjusted $P = 0.009$; TG/GG vs. TT: adjusted OR = 0.76, 95% CI = 0.61-0.95, adjusted $P = 0.018$). No association was observed for rs3203713 variant genotypes and risk of SCCHN in both subgroups. In addition, the locus-dose effect of combined risk alleles was also seen in SCCHN arising at non-oropharyngeal sites ($P_{\text{trend}} = 0.017$). When the group of “0-1” risk allele was used as the reference, the group with “2-6” risk alleles had a significantly higher risk of SCCHN arising at non-oropharyngeal sites (adjusted OR = 1.35, 95% CI = 1.03-1.78; adjusted $P = 0.033$). However, these associations were not found for oropharyngeal cancer.

Haplotype and stratification analyses

We further evaluated the combined effect of the three polymorphisms on risk of SCCHN arising at non-oropharyngeal sites by using the haplotype analysis (Table 3). A total of six haplotypes were derived from the observed genotypes, of which T_A11599672_A2274223_A3203713 was the most common haplotype in cases and controls with the
frequencies of 53.6% and 53.0%, respectively. Compared with the common haplotype, the Grs11599672 A rs2274223 A rs3203713 haplotype was associated with a 28% decreased risk of SCCHN arising at non-oropharyngeal sites (adjusted OR = 0.72, 95% CI = 0.56-0.92); in contrast, the Trs11599672G rs2274223A rs3203713 haplotype was associated with a 31% increased risk of SCCHN arising at non-oropharyngeal sites (adjusted OR = 1.31, 95% CI = 1.00-1.72). These associations were not observed for other haplotypes.

We then performed stratification analyses to evaluate the effects of variant genotypes on risk of SCCHN arising at non-oropharyngeal sites by age, sex, smoking status, alcohol use and disease stage (Table 4). The results showed that the risk effect of rs2274223 was more evident in the younger (adjusted OR = 1.44, 95% CI = 1.02-2.02), male (adjusted OR = 1.51, 95% CI = 1.14-2.00), smokers (adjusted OR = 1.33, 95% CI = 1.02-1.72), drinkers (adjusted OR = 1.69, 95% CI = 1.25-2.28)...

Table 2 Logistic regression analysis for associations between PLCE1 variant genotypes and SCCHN risk

| Locus      | Genotype | Controls (%) | Overall (N = 1,098) | Oropharynx (N = 559) | Non-oropharynx (N = 539) | OR (95%CI) |
|------------|----------|--------------|---------------------|----------------------|--------------------------|------------|
| PLCE1 rs2274223 | AA       | 504 (46.3)   | 477 (43.5)          | 1.00                 | 253 (45.3)                | 1.00       |
|            | AG       | 474 (43.5)   | 506 (46.1)          | 1.14 (0.95-1.38)     | 248 (44.4)                | 1.09 (0.87-1.36) |
|            | GG       | 111 (10.2)   | 114 (10.4)          | 1.20 (0.98-1.52)     | 58 (10.3)                 | 1.15 (0.80-1.64) |
|            | AG/GG    | 585 (53.7)   | 620 (56.5)          | 1.15 (0.97-1.38)     | 306 (54.7)                | 1.10 (0.89-1.36) |
| G allele   | 0.320    |              | 0.335               |                      |                          |            |

Table 3 PLCE1 haplotype and risk of SCCHN arising at non-oropharyngeal sites

| Haplotype frequencies | Cases (N = 1,098) | Controls (N = 2,134) | Crude OR (95% CI) | Adjusted OR b (95% CI) | P b |
|-----------------------|------------------|----------------------|-------------------|------------------------|-----|
| TAA                   | 572              | 53.6                 | 1131              | 53.0                   | 1.00| 1.00 |
| GAA                   | 128              | 12.0                 | 318               | 14.9                   | 0.80 (0.63-1.00) | 0.72 (0.56-0.92) | 0.008|
| TGA                   | 119              | 11.1                 | 198               | 93                     | 1.19 (0.99-1.35) | 1.31 (1.00-1.72) | 0.049|
| GGG                   | 87               | 8.1                  | 179               | 8.4                    | 0.96 (0.73-1.27) | 0.96 (0.71-1.39) | 0.771|
| TGG                   | 96               | 9.0                  | 170               | 8.0                    | 1.12 (0.85-1.46) | 1.10 (0.82-1.46) | 0.528|
| GGA                   | 66               | 6.2                  | 138               | 6.5                    | 0.95 (0.69-1.29) | 0.85 (0.61-1.18) | 0.334|

a The alleles of haplotypes were arrayed as the location of the SNPs in PLCE1 stand from 5’ to 3’ (e.g. TAA denotes T_rs11599672A, rs2274223A, rs3203713A).
b Adjusted for age, gender, smoking and alcohol status in logistic models.
| Variables                  | rs2274223 (cases/ controls) | Adjusted OR* (95%CI) | rs11599672 (cases/ controls) | Adjusted OR* (95%CI) | Combined effect of risk alleles b (cases/ controls) | Adjusted OR* (95%CI) |
|---------------------------|------------------------------|----------------------|-----------------------------|----------------------|---------------------------------------------------|---------------------|
|                           | AA                           | AG/GG                | TT                          | TG/GG                | 0-1                                               | 2-6                 |
| Age, yr                   |                              |                      |                             |                      |                                                   |                     |
| ≤57(median)               | 96/286                       | 133/285              | 1.44 (1.02-2.02)            | 133/284              | 97/279                                           | 0.71 (0.50-1.00)    |
| >57(median)               | 128/218                      | 181/300              | 1.19 (0.87-1.63)            | 151/235              | 156/276                                           | 0.88 (0.64-1.20)    |
| Gender                    |                              |                      |                             |                      |                                                   |                     |
| Females                   | 84/119                       | 100/139              | 0.97 (0.64-1.47)            | 96/110               | 89/145                                           | 0.73 (0.48-1.10)    |
| Males                     | 140/385                      | 214/446              | 1.51 (1.14-2.00)            | 188/409              | 164/410                                           | 0.84 (0.64-1.10)    |
| Smoking status            |                              |                      |                             |                      |                                                   |                     |
| Never                     | 46/236                       | 57/298               | 0.99 (0.65-1.53)            | 54/266               | 48/261                                           | 0.85 (0.55-1.31)    |
| Ever                      | 178/268                      | 257/287              | 1.33 (1.02-1.72)            | 230/253              | 205/294                                           | 0.73 (0.56-0.94)    |
| Alcohol status            |                              |                      |                             |                      |                                                   |                     |
| Never                     | 79/210                       | 76/262               | 0.84 (0.57-1.23)            | 86/223               | 69/244                                           | 0.67 (0.46-0.99)    |
| Ever                      | 145/294                      | 238/323              | 1.69 (1.25-2.28)            | 198/296              | 184/311                                           | 0.86 (0.64-1.15)    |
| Stage                     |                              |                      |                             |                      |                                                   |                     |
| I-II                      | 87/504                       | 137/585              | 1.40 (1.03-1.91)            | 114/519              | 111/555                                           | 0.84 (0.62-1.14)    |
| III-IV                    | 137/504                      | 177/585              | 1.24 (0.94-1.65)            | 170/519              | 142/555                                           | 0.78 (0.59-1.03)    |

* Adjusted for age, sex, smoking and alcohol status (the stratified factor in each stratum excluded).

b The risk alleles: rs2274223G, rs3203713G and rs11599672T.
and subjects with early stage (adjusted OR = 1.40, 95% CI = 1.03-1.91), whereas the protective effect of rs11599672 was more prominent in the younger (adjusted OR = 0.71, 95% CI = 0.50-1.00), smokers (adjusted OR = 0.73, 95% CI = 0.56-0.94) and non-drinkers (adjusted OR = 0.67, 95% CI = 0.46-0.99). Additionally, the increased risk associated with the combined effect of risk alleles was also more pronounced among the younger (adjusted OR = 1.68, 95% CI = 1.09-2.59), male (adjusted OR = 1.41, 95% CI = 1.00-1.98), smokers (adjusted OR = 1.65, 95% CI = 1.21-2.26), drinkers (adjusted OR = 1.42, 95% CI = 1.00-2.03) and subjects with early-stage tumors (adjusted OR = 1.46, 95% CI = 1.01-2.13). However, no significant associations were found for rs3203713 variant genotypes and risk of SCCHN arising at non-oropharyngeal sites in every stratum (data not shown). Heterogeneity test showed that there was no significant heterogeneity (\( P > 0.05 \)) in every two strata except for that of rs2274223 stratified by alcohol status (\( P = 0.005 \)). Interestingly, we detected a significant interaction between alcohol status (never and ever) and rs2274223 variant genotypes (AA and AG/GG) for risk of SCCHN arising at non-oropharyngeal sites, even after the Bonferroni correction (\( P_{\text{int}} = 0.004 \)).

**Discussion**

In this case-control study, we investigated the associations between three novel, potentially functional SNPs of *PLCE1* (rs2274223, rs3203713 and rs11599672) and risk of SCCHN in a non-Hispanic white population. Although we did not find evidence of a main effect of each *PLCE1* SNP on overall SCCHN risk, the joint effect of these variants appeared to contribute to risk of SCCHN in a dose-response manner, especially for cancers arising at non-oropharyngeal sites. Further, the subgroup analysis of SCCHN arising at non-oropharyngeal sites showed that variant genotypes of both rs2274223 and rs11599672 were independently associated with the risk. These findings suggested, for the first time, that potentially functional polymorphisms of *PLCE1* may play a role in the development of SCCHN, particularly of those tumors at non-oropharyngeal sites.

*PLCE1* is known to be a direct downstream effector of small GTPases (Ras, Rap1 and Rap2), with the presence of two Ras-associating domains at its C terminus and a CDC25 guanine exchange factor (CDC25 gef) domain at its N terminus [11,23-25]. Since mutations in the RAS gene family are associated with about 30% of all human cancers, several studies have investigated the possible role of PLCE1 in cancer development and progression [13-16,26]. It has been reported that *PLCE1* has an onco-

inflammation, binding to the Ras family small GTPase, and augmentation of angiogenesis [13-16,26]. Recent evidence has demonstrated that *PLCE1* mutations might cause the nephritic syndrome [23] and diffuse mesangial sclerosis (DMS) [27]; however, few studies have investigated the association between genetic variants of *PLCE1* and risk of human cancers.

Most recently, for the first time, Wang *et al.* found that rs2274223, a nonsynonymous SNP of the *PLCE1* gene, was associated with an increased risk of ESCC and GCA in a Chinese population by a two-stage GWAS in 9,053 ESCC cases, 2,766 GCA cases and 11,013 controls [8]. Likewise, in another GWAS including 2,115 ESCC cases, 2,240 gastric cancer cases and 3,302 controls in another Chinese population, Abnet *et al.* also reported that rs2274223 was a notable signal for susceptibility to ESCC and GCA [10]. Despite the present study with 1,098 SCCHN cases and 1,090 controls may have a limited power to detect weak associations between polymorphisms of *PLCE1* including rs2274223 and overall risk of SCCHN compared with the published GWA studies [9,10], our results did show that subjects carrying more risk alleles in *PLCE1* (rs2274223G, rs3203713G and rs11599672T) had a higher risk of SCCHN than those with zero to one risk allele, especially for SCCHN arising at non-oropharyngeal sites, suggesting a joint effect of these SNPs on susceptibility to SCCHN. Given only a modest effect of each SNP individually, evaluating their combined effects may help us better understand any role of *PLCE1* SNPs in cancer etiology. Furthermore, different carcinogenic mechanisms between esophageal and gastric cancers and SCCHN or genetic difference in different populations may result in the discrepancy for the main effect of rs2274223 between our study on SCCHN and two GWASs of esophageal and gastric cancers.

Studies have shown that the oropharynx is the most common site for HPV-associated SCCHN [28,29]; in contrast, SCCHN arising at non-oropharyngeal sites have much lower HPV seroprevalence [28,30] and thus are more likely to be caused by smoking and alcohol drinking, similar to ESCC and GCA. In fact, 36% of our oropharyngeal cancer patients were never smokers compared to only 19% of patients with SCCHN arising at non-oropharyngeal sites. In our study, we found that rs2274223 and rs11599672 had an independent effect on risk of smoking and drinking related SCCHN arising at non-oropharyngeal sites but not HPV-related oropharyngeal cancer. Even after Bonferroni corrections, the association of rs11599672 with non-oropharyngeal SCCHN and association of rs2274223 with drinking at non-oropharyngeal sites remained significant, suggesting different roles of these polymorphisms in the etiology of two different tumor sub-sites. Additionally, subgroup analyses restricted to oropharyngeal cancer did find a similar pattern of risk (though
that rs3203713 in PLCE1 binding site (TFBS) of PLCE1. Further, rs11599672 is located in the transcription factor affecting the binding of miRNA and target gene.

We found that three PLCE1 SNPs may have a joint effect on the risk of SCCHN, especially those arising at non-oropharyngeal sites, including oral cavity, hypopharynx or larynx, associated with smoking and alcohol exposure. Furthermore, those results from subgroup analysis suggest that variant genotypes of rs2274223 and rs11599672 independently affect the risk of SCCHN arising at non-oropharyngeal sites. Additional larger studies in different populations are needed to validate our findings.

Conclusions

In summary, in this hospital-based case-control study, we found that three PLCE1 SNPs may have a joint effect on the risk of SCCHN, especially those arising at non-oropharyngeal sites, including oral cavity, hypopharynx or larynx, associated with smoking and alcohol exposure. Furthermore, those results from subgroup analysis suggest that variant genotypes of rs2274223 and rs11599672 may independently affect the risk of SCCHN arising at non-oropharyngeal sites. Additional larger studies in different populations are needed to validate our findings.

Abbreviations

OR: odds ratio; CI: confidence interval; PLCE1: phospholipase C epsilon 1 gene; GWAS: genome-wide association study; LD: linkage disequilibrium; MAF: minor allele frequency; PCR: polymerase chain reaction; SCCHN: squamous cell carcinoma of the head and neck; TFBS: transcription factor binding site

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Authors’ contributions

HXM, LEW and QYW designed the study and wrote the manuscript. HXM performed the experiments with the assistance from ZSL. EMS helped to revise the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.
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