

Replication study of *PLCE1* and *C20orf54* polymorphism and risk of esophageal cancer in a Chinese population

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Abstract Esophageal cancer is one of the most aggressive cancers in the world. Recent large-scale genome-wide association studies (GWAS) reported that functional genetic variations in the phospholipase C epsilon gene (*PLCE1*) were strongly associated with risk of esophageal squamous cell carcinoma (ESCC) and gastric cardia adenocarcinoma (GCA) in Chinese population. For *C20orf54* rs13042395 genotype and risk of esophageal cancer, the results were inconsistent. We conducted a replication case–control study to evaluate the genetic effects of these two functional single nucleotide polymorphisms (SNPs) on the development of esophageal cancer. A total of 380 cases and 380 controls were recruited for this study. The genotypes were determined by matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-ToF MS). The variant alleles of the functional polymorphism, *PLCE1* rs2274223 SNP was associated with the increased risk of esophageal cancer [adjusted odds ratio (OR) = 1.95, 95 % confidence interval (CI) = 1.05–3.59 for *PLCE1* rs2274223 GG vs. AA]. However, there was no significant association between the *C20orf54* rs13042395 genotype and esophageal cancer risk

(adjusted OR = 0.99, 95 % CI = 0.63–1.57 for *C20orf54* rs13042395 TT vs. CC). Stratified analyses indicated a significantly increased risk of esophageal cancer associated with the *PLCE1* rs2274223 AG genotype was more evident among females, younger patients and never drinkers, compared with the *PLCE1* rs2274223 AA genotypes. Stratified analyses also indicated a significantly increased risk of esophageal cancer associated with the *PLCE1* rs2274223 GG genotype was more evident among never smokers and never drinkers compared with the *PLCE1* rs2274223 AA genotypes. These findings indicated that functional polymorphisms *PLCE1* rs2274223 might contribute to esophageal cancer susceptibility.

Keywords *PLCE1*, *C20orf54* · Polymorphisms · Esophageal cancer · Molecular epidemiology

Abbreviations

CI	Confidential interval
GWAS	Genome-wide association study
LD	Linkage disequilibrium
OR	Odds ratio
SNPs	Single nucleotide polymorphisms

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Introduction

Esophageal cancer is one of ten most common cancers in the world [1]. ESCC is the most frequent subtype of esophageal cancer and accounts for >90 % of cases [2]. Epidemiological studies indicate that tobacco and alcohol are the major risk factors for esophageal cancer. However, only a subset of individuals exposed to the environmental risk factors would develop esophageal cancer, suggesting a role of host factors

and genetic alterations in esophageal cancer carcinogenesis through gene-environment interactions. Some studies have suggested that genetic polymorphisms might explain individual differences in susceptibility to esophageal cancer [3].

The *PLCE1* gene is located on chromosome 10q23 [4]. Mutations in *PLCE1* have been related with isolated diffuse mesangial sclerosis and some rare cases of focal segmental glomerulosclerosis [4, 5]. The *PLCE1* gene encodes phospholipase C epsilon 1 (PLC ϵ 1), which catalyzes the hydrolysis of polyphosphoinositides to generate second messengers, such as inositol-1, 4, 5 trisphosphate and diacylglycerol (DAG) [6]. PLC ϵ 1 may be related to cellular differentiation and apoptosis through its coaction with Ras family [7, 8]. Studies have reported that PLC ϵ 1 plays crucial roles in carcinogenesis and progression of different types of cancers [9–13].

In 2010, two large-scale genome-wide association studies simultaneously reported that a new and notable low-penetrance susceptibility locus (rs2274223) located in *PLCE1* was strongly associated with risk of ESCC and GCA in Chinese population [3, 14]. Additionally, one study also showed that the positive rates of the PLC ϵ 1 protein in ESCC and GCA tissues were significantly higher than that in normal ones, suggesting a biological role of PLC ϵ 1 in ESCC and GCA carcinogenesis [3]. It has been identified that rs2274223 is a non-synonymous SNP of *PLCE1*, causing the amino acid change from histidine to arginine. Another SNP rs13042395 was localized to 5' flanking region of chromosome 20 open reading frame 54 (*C20orf54*). For *C20orf54* rs13042395 genotype and risk of esophageal cancer, the results were inconsistent. One genome-wide association study showed positive result while another didn't find the association between this polymorphism and esophageal cancer risk [3, 14].

On the basis of the biological and pathologic significance of *PLCE1* and *C20orf54*, it is possible that functional genetic variations in the *PLCE1* and *C20orf54* gene may contribute to the development of esophageal cancer. The objective of this investigation was to evaluate the association between *PLCE1* and *C20orf54* genotypes and esophageal cancer susceptibility in a hospital-based case-control study. We performed genotyping analyses for the two SNPs with 380 ESCC cases and 380 controls in a Chinese population.

Patients 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). All subjects provided written informed consent to be included in the study.

Study subjects

Three-hundred and eighty subjects with esophageal cancer were consecutively recruited from the Affiliated People's Hospital of Jiangsu University and Affiliated Hospital of Jiangsu University (Jiangsu, China) between October 2008 and November 2009. All cases of esophageal cancer were diagnosed as ESCC by pathologic means. The exclusion criteria were patients who previously had: cancer; any metastasized cancer; radiotherapy or chemotherapy. The 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 these control subjects had trauma or infectious diseases.

Each subject was personally questioned by trained interviewers using a pre-tested questionnaire to obtain information on demographic data (e.g., age, sex) and related risk factors (including tobacco smoking and alcohol consumption). After the interview, 2 mL samples of venous blood were collected from each subject. 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 matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-ToF-MS)

Blood samples were collected from patients using Vacutainers and transferred to tubes lined with ethylenediamine tetraacetic acid (EDTA). Genomic DNA was isolated from whole blood with the QIAamp DNA Blood Mini Kit (Qiagen, Berlin, Germany). Genotyping was undertaken by MALDI-ToF-MS as previously described [15]. SNP genotyping was done using the MassArray system (Sequenom, San Diego, California) by MALDI-ToF-MS according to manufacturer's instructions. Completed genotyping reactions were spotted onto a 384-well spectroCHIP system (Sequenom) using a MassArray Nanodispenser (Sequenom) and determined by MALDI-ToF-MS. Genotype calling was done in real time with MassArray RT software version 3.1 (Sequenom), and analyzed using MassARRAY Typer software version 4.0 (Sequenom) (Figs. 1, 2). For quality control, repeated analyses were done for 10 % of randomly selected samples.

Statistical analyses

Differences in the distributions of demographic characteristics, selected variables, and genotypes of the *PLCE1* rs2274223 and *C20orf54* rs13042395 variants between the cases and controls were evaluated using the χ^2 test. The

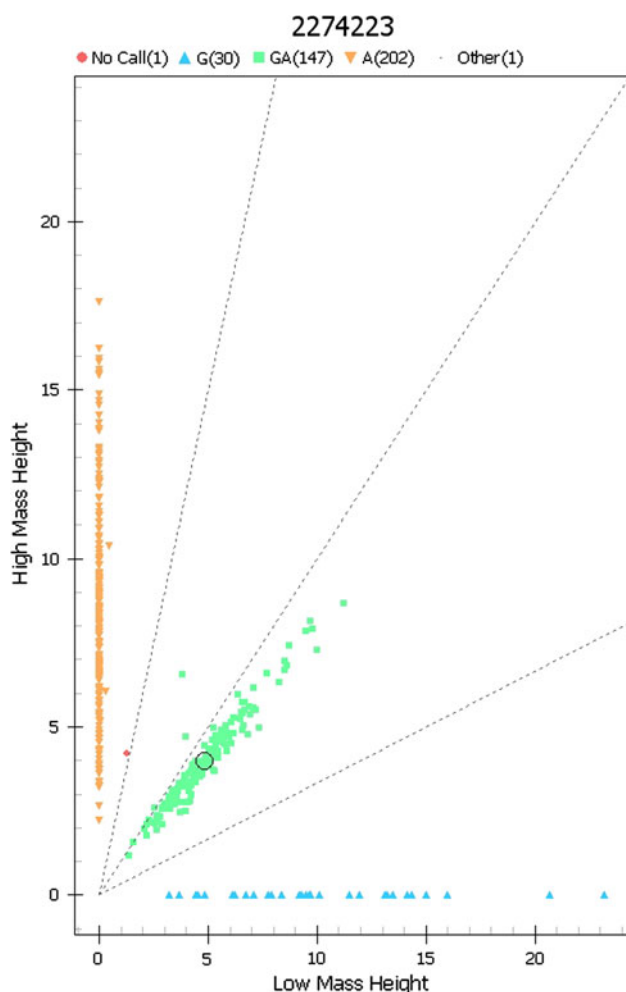


Fig. 1 Genotyping of *PLCE1* rs2274223 A/G by MALDI-ToF-MS

associations between *PLCE1* rs2274223 and *C20orf54* rs13042395 genotypes and risk of esophageal cancer were estimated by computing the ORs and their 95 % CIs using logistic regression analyses for crude ORs and adjusted ORs when adjusting for age, sex, smoking and drinking status. The Hardy–Weinberg equilibrium was tested by a goodness-of-fit χ^2 test to compare the observed genotype frequencies to the expected ones among the control subjects. All statistical analyses were performed with SAS 9.1.3 (SAS Institute, Cary, NC, USA).

Results

Characteristics of the study population

Among the 380 cases and 380 controls with DNA samples, the genotyping was successful in 379 (99.7 %) cancer cases, and 371 (97.6 %) controls for *PLCE1* rs2274223. For *C20orf54* rs13042395, the genotyping was successful in 379

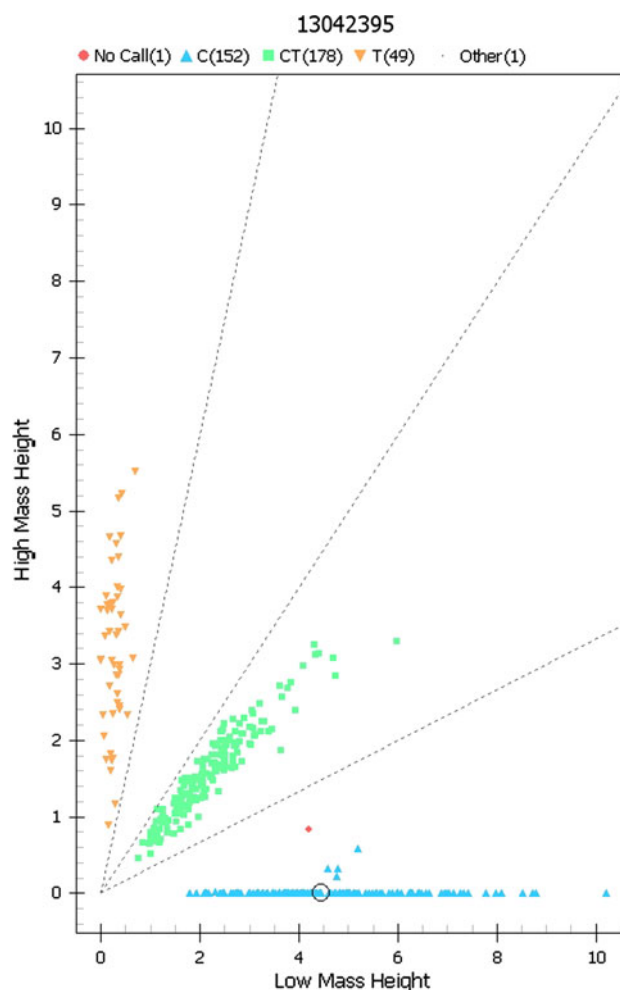


Fig. 2 Genotyping of *C20orf54* rs13042395 C/T by MALDI-ToF-MS

(99.7 %) cancer cases and 375 (98.7 %) controls. Characteristics of cases and controls included in the study are summarized in Table 1. The cases and controls appeared to be adequately matched on age and sex as suggested by the χ^2 tests ($p = 0.056$ and $p = 0.346$, respectively). As shown in Table 1, no significant difference was detected on drinking status between the cases and the controls ($p = 0.183$), but smoking rate was higher in esophageal cancer patients than in control subjects ($p = 0.014$).

Associations between *PLCE1* rs2274223 and *C20orf54* rs13042395 polymorphisms and risk of esophageal cancer

The genotype distributions of *PLCE1* rs2274223 and *C20orf54* rs13042395 in the cases and the controls are shown in Table 2. The observed genotype frequencies for these two polymorphisms in the controls were all in Hardy–Weinberg equilibrium ($p = 0.457$ and 0.648 for *PLCE1* rs2274223 and *C20orf54* rs13042395, respectively). In the single locus

Table 1 Distribution of selected demographic variables and risk factors in esophageal cancer cases and controls

Variable	Cases (<i>n</i> = 380)		Controls (<i>n</i> = 380)		<i>p</i> value*
	<i>n</i>	%	<i>n</i>	%	
Age (years)					0.056
<60	142	37.4	117	30.8	
≥60	238	62.6	263	69.2	
Sex					0.346
Male	269	70.8	257	67.6	
Female	111	29.2	123	32.4	
Tobacco use					0.014
Never	220	57.9	253	66.6	
Ever	160	42.1	127	33.4	
Alcohol use					0.183
Never	253	66.6	270	71.1	
Ever	127	33.4	110	28.9	

* Two-sided χ^2 test

analyses, the genotype frequencies of *PLCE1* rs2274223 were 53.3 % (AA), 38.8 % (AG), and 7.9 % (GG) in the case patients and 62.8 % (AA), 32.1 % (AG), and 5.1 % (GG) in the control subjects, and the difference was statistically significant ($p = 0.023$). Logistic regression analyses revealed that subjects carrying the *PLCE1* rs2274223 GG variant homozygote had a significantly 1.95-fold (adjusted OR = 1.95; 95 % CI = 1.05–3.59) increased risk

of esophageal cancer, compared with the *PLCE1* rs2274223 AA wide-type homozygote. However, the genotype frequencies of *C20orf54* rs13042395 were not significantly different between the cases and the controls ($p = 0.991$). Logistic regression analyses revealed that the both *C20orf54* rs13042395 TT variant genotype and the *C20orf54* rs13042395 CT heterozygote were not associated with the risk of esophageal cancer (adjusted OR = 0.99, 95 % CI = 0.63–1.57 for rs13042395 TT and adjusted OR = 0.97, 95 % CI = 0.71–1.33 for rs13042395 CT, respectively), compared with the *C20orf54* rs13042395 CC wide-type homozygote.

In the dominant model, the *PLCE1* rs2274223 AG/GG variants were associated with increased risk of esophageal cancer, compared with the *PLCE1* rs2274223 AA genotype (adjusted OR = 1.51, 95 % CI = 1.12–2.03). For the *C20orf54* rs13042395 C/T polymorphism, the *C20orf54* rs13042395 CT/TT genotypes were not associated with the risk of esophageal cancer (adjusted OR = 0.98, 95 % CI = 0.73–1.31), compared with the *C20orf54* rs13042395 CC genotype.

In the recessive model, the *PLCE1* rs2274223 GG variant homozygote was not associated with the risk of esophageal cancer, compared with the *PLCE1* rs2274223 AA/AG genotypes (adjusted OR = 1.69, 95 % CI = 0.93–3.08). For the *C20orf54* rs13042395 C/T polymorphism, the *C20orf54* rs13042395 TT genotype was not associated with

Table 2 Logistic regression analyses of associations between *PLCE1* rs2274223 and *C20orf54* rs13042395 polymorphisms and risk of esophageal cancer

Genotype	Cases (<i>n</i> = 380) ^a		Controls (<i>n</i> = 380)		Crude OR (95 % CI)	<i>p</i>	Adjusted OR ^b (95 % CI)	<i>p</i>
	<i>n</i>	%	<i>n</i>	%				
<i>PLCE1</i> rs2274223								
AA	202	53.3	233	62.8	1.00		1.00	
AG	147	38.8	119	32.1	1.43 (1.05–1.94)	0.024	1.44 (1.06–1.96)	0.020
GG	30	7.9	19	5.1	1.82 (1.00–3.33)	0.052	1.95 (1.05–3.59)	0.034
AG + GG	177	46.7	138	37.2	1.48 (1.11–1.98)	0.009	1.51 (1.12–2.03)	0.006
AA + AG	349	92.1	352	94.9	1.00		1.00	
GG	30	7.9	19	5.1	1.59 (0.88–2.88)	0.124	1.69 (0.93–3.08)	0.088
G allele		27.3		21.2				
<i>C20orf54</i> rs13042395								
CC	152	40.1	149	39.7	1.00		1.00	
CT	178	47.0	178	47.5	0.98 (0.72–1.33)	0.899	0.97 (0.71–1.33)	0.865
TT	49	12.9	48	12.8	1.00 (0.63–1.58)	0.998	0.99 (0.63–1.57)	0.970
CT + TT	227	59.9	226	60.3	0.99 (0.74–1.32)	0.917	0.98 (0.73–1.31)	0.878
CC + CT	330	87.1	327	87.2	1.00		1.00	
TT	49	12.9	48	12.8	1.01 (0.66–1.55)	0.958	1.01 (0.66–1.54)	0.979
T allele		36.4		36.5				

^a The genotyping was successful in 379 (99.7 %) cancer cases, and 371 (97.6 %) controls for *PLCE1* rs2274223. For *C20orf54* rs13042395, the genotyping was successful in 379 (99.7 %) cancer cases and 375 (98.7 %) controls

^b Adjusted for age, sex, smoking and drinking status

Both *C20orf54* rs13042395 TT variant genotype and the *C20orf54* rs13042395 CT heterozygote were not associated with the risk of esophageal cancer after stratification.

Discussion

Mutational activation of the ras proto-oncogenes is frequently found in cancers. PLC ϵ 1 may also function as a Ras receptor and could play a role in promoting apoptosis, thereby acting as a tumor suppressor [20]. PLC ϵ 1 may be

Table 3 Stratified analyses between *PLCE1* rs2274223 and *C20orf54* rs13042395 polymorphisms and esophageal cancer risk by sex, age, smoking status and alcohol consumption

Variable	PLCE1 rs2274223 (case/control) ^a			OR ^b (95 % CI)			C20orf54 rs13042395 (case/control)			OR ^b (95 % CI)		
	AA	AG	GG	AA	AG	GG	CC	CT	TT	CC	CT	TT
Sex												
Male	153/159	102/82	14/9	1.00	1.31(0.91–1.90)	1.50(0.62–3.63)	112/112	118/114	39/29	1.00	1.00(0.69–1.45)	1.36(0.78–2.37)
Female	49/74	45/37	16/10	1.00	1.85(1.04–3.27)	2.35(0.98–5.62)	40/37	60/64	10/19	1.00	0.89(0.50–1.57)	0.51(0.21–1.26)
Age												
<60	74/76	54/28	14/8	1.00	1.85(1.04–3.31)	1.89(0.72–4.91)	59/47	65/58	18/10	1.00	0.77(0.44–1.33)	1.36(0.55–3.35)
≥60	128/157	93/91	16/11	1.00	1.24(0.85–1.80)	1.96(0.87–4.44)	93/102	113/120	31/38	1.00	1.05(0.72–1.54)	0.91(0.52–1.59)
Smoking status												
Never	111/150	86/82	22/13	1.00	1.40(0.94–2.08)	2.26(1.08–4.74)	92/98	100/119	27/32	1.00	0.90(0.61–1.35)	0.89(0.49–1.61)
Ever	91/83	61/37	8/6	1.00	1.41(0.83–2.38)	1.05(0.32–3.42)	60/51	78/59	22/16	1.00	1.16(0.68–1.97)	1.26(0.58–2.75)
Alcohol consumption												
Never	130/162	100/86	22/14	1.00	1.46(1.00–2.13)	2.11(1.03–4.35)	106/106	114/127	32/33	1.00	0.90(0.62–1.32)	0.97(0.55–1.70)
Ever	72/71	47/33	8/5	1.00	1.20(0.67–2.18)	1.23(0.35–4.32)	46/43	64/51	17/15	1.00	1.26(0.69–2.29)	1.11(0.47–2.65)

^a The genotyping was successful in 379 (99.7 %) cancer cases, and 371 (97.6 %) controls for *PLCE1* rs2274223. For *C20orf54* rs13042395, the genotyping was successful in 379 (99.7 %) cancer cases and 375 (98.7 %) controls

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

involved in the development of sporadic colorectal cancer through its inhibitory effect on cell proliferation [20]. Overexpression of *PLCε1* significantly inhibited the proliferation of colon cancer cells and degraded its malignant degree [20]. *PLCE1* rs2274223 is a non-synonymous SNP with A to G transition in exon 26, which results in a substitution of histidine to arginine. Wang et al. [3] found that *PLCE1* rs2274223 was associated with an increased risk of ESCC. Abnet et al. [14] also reported that *PLCE1* rs2274223 was a notable signal for susceptibility to ESCC, which is consistent with our results.

There was no significant association between the *C20orf54* rs13042395 genotype and esophageal cancer risk in our population. These findings were not consistent with one recent GWAS identified the variation of *C20orf54* rs13042395 as risk factors for esophageal cancer in Chinese population [3]. However, in a different Chinese population, another GWAS found negative result, which is in accordance with our research [14]. In a previous research, no significant association was detected between *C20orf54* rs13042395 at 20p13 and gastric cancer risk [21].

The frequencies of genetic polymorphisms often vary between ethnic groups. In the present Chinese study, the allele frequency of *PLCE1* rs2274223 G was 0.212 among 380 control subjects, which is consistent with that of Chinese Han population (0.20 among 1,733 control subjects of GWAS samples, 0.22 among 11,013 control subjects of ESCC replication 1 in GWAS by Wang et al. and 0.209 in another 3,302 controls combined in GWAS by Abnet et al. [3, 14]) but significantly lower than that of non-Hispanic whites (0.320 among 1,090 control subjects) [19].

In this case–control study, several limitations need to be addressed. Firstly, we enrolled the patients and controls from the hospitals and the study subjects may not be representative of the general population, inherited biases may lead to spurious findings. Secondly, the polymorphisms investigated in our study, based on their functional considerations, may not give a comprehensive view about genetic variability in *PLCE1* and *C20orf54*. Further studies by fine-mapping the susceptible region of the variants are needed to clarify the genetic mechanism of esophageal carcinogenesis. Thirdly, the moderate sample size limited the statistical power of our study. With the sample size of 380 cases and 380 controls, we have an 80 % statistical power to test the lowest OR of 1.598(0.581) for *PLCE1* rs2274223 and 1.512(0.645) for *C20orf54* rs13042395. Large well-designed studies are warranted to confirm our findings, particularly the gene–environment interaction. Finally, we did not obtain a detailed information on cancer metastasis and survival information, which restricted us from further analyses on the role of *PLCE1* and *C20orf54* polymorphisms in esophageal cancer progression and prognosis.

In conclusion, our study provides strong evidence that functional polymorphism of *PLCE1* rs2274223 may contribute to the risk of esophageal cancer. However, our results were obtained with a limited sample size and therefore allowed us to draw just preliminary conclusions. Validation of these findings with functional evaluation and larger studies with more rigorous study designs of other ethnic populations are needed.

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References

- Shinomiya T, Mori T, Ariyama Y, Sakabe T, Fukuda Y, Murakami Y, Nakamura Y, Inazawa J (1999) Comparative genomic hybridization of squamous cell carcinoma of the esophagus: the possible involvement of the DPI gene in the 13q34 amplicon. *Genes Chromosomes Cancer* 24:337–344
- Wu C, Hu Z, He Z, Jia W, Wang F, Zhou Y, Liu Z, Zhan Q, Liu Y, Yu D, Zhai K, Chang J, Qiao Y, Jin G, Liu Z, Shen Y, Guo C, Fu J, Miao X, Tan W, Shen H, Ke Y, Zeng Y, Wu T, Lin D (2011) Genome-wide association study identifies three new susceptibility loci for esophageal squamous-cell carcinoma in Chinese populations. *Nat Genet* 43:679–684
- Wang LD, Zhou FY, Li XM, Sun LD, Song X, Jin Y, Li JM, Kong GQ, Qi H, Cui J, Zhang LQ, Yang JZ, Li JL, Li XC, Ren JL, Liu ZC, Gao WJ, Yuan L, Wei W, Zhang YR, Wang WP, Sheyhidin I, Li F, Chen BP, Ren SW, Liu B, Li D, Ku JW, Fan ZM, Zhou SL, Guo ZG, Zhao XK, Liu N, Ai YH, Shen FF, Cui WY, Song S, Guo T, Huang J, Yuan C, Huang J, Wu Y, Yue WB, Feng CW, Li HL, Wang Y, Tian JY, Lu Y, Yuan Y, Zhu WL, Liu M, Fu WJ, Yang X, Wang HJ, Han SL, Chen J, Han M, Wang HY, Zhang P, Li XM, Dong JC, Xing GL, Wang R, Guo M, Chang ZW, Liu HL, Guo L, Yuan ZQ, Liu H, Lu Q, Yang LQ, Zhu FG, Yang XF, Feng XS, Wang Z, Li Y, Gao SG, Qige Q, Bai LT, Yang WJ, Lei GY, Shen ZY, Chen LQ, Li EM, Xu LY, Wu ZY, Cao WK, Wang JP, Bao ZQ, Chen JL, Ding GC, Zhuang X, Zhou YF, Zheng HF, Zhang Z, Zuo XB, Dong ZM, Fan DM, He X, Wang J, Zhou Q, Zhang QX, Jiao XY, Lian SY, Ji AF, Lu XM, Wang JS, Chang FB, Lu CD, Chen ZG, Miao JJ, Fan ZL, Lin RB, Liu TJ, Wei JC, Kong QP, Lan Y, Fan YJ, Gao FS, Wang TY, Xie D, Chen SQ, Yang WC, Hong JY, Wang L, Qiu SL, Cai ZM, Zhang XJ (2010) Genome-wide association study of esophageal squamous cell carcinoma in Chinese subjects identifies susceptibility loci at *PLCE1* and *C20orf54*. *Nat Genet* 42:759–763
- Hinkes B, Wiggins RC, Gbadegesin R, Vlangos CN, Seelow D, Nurnberg G, Garg P, Verma R, Chaib H, Hoskins BE, Ashraf S, Becker C, Hennies HC, Goyal M, Wharram BL, Schachter AD, Mudumana S, Drummond I, Kerjaschki D, Waldherr R, Dietrich A, Ozaltin F, Bakkaloglu A, Cleper R, Basel-Vanagaite L, Pohl M, Griebel M, Tsygin AN, Soyulu A, Muller D, Sorli CS, Bunney TD, Katan M, Liu J, Attanasio M, O'Toole JF, Hasselbacher K, Mucha B, Otto EA, Airik R, Kispert A, Kelley GG, Smrcka AV, Gudermann T, Holzman LB, Nurnberg P, Hildebrandt F (2006) Positional cloning uncovers mutations in *PLCE1* responsible for a nephrotic syndrome variant that may be reversible. *Nat Genet* 38:1397–1405
- Gbadegesin R, Hinkes BG, Hoskins BE, Vlangos CN, Heeringa SF, Liu J, Loirat C, Ozaltin F, Hashmi S, Ulmer F, Cleper R, Ettenger R,

- Antignac C, Wiggins RC, Zenker M, Hildebrandt F (2008) Mutations in PLCE1 are a major cause of isolated diffuse mesangial sclerosis (IDMS). *Nephrol Dial Transplant* 23:1291–1297
6. Wing MR, Bourdon DM, Harden TK (2003) PLC-epsilon: a shared effector protein in Ras-, Rho-, and G alpha beta gamma-mediated signaling. *Mol Interv* 3:273–280
 7. Harden TK, Sondek J (2006) Regulation of phospholipase C isozymes by ras superfamily GTPases. *Annu Rev Pharmacol Toxicol* 46:355–379
 8. Ada-Nguema AS, Xenias H, Hofman JM, Wiggins CH, Sheetz MP, Keely PJ (2006) The small GTPase R-Ras regulates organization of actin and drives membrane protrusions through the activity of PLCepsilon. *J Cell Sci* 119:1307–1319
 9. Li M, Edamatsu H, Kitazawa R, Kitazawa S, Kataoka T (2009) Phospholipase cepsilon promotes intestinal tumorigenesis of Apc(Min/+) mice through augmentation of inflammation and angiogenesis. *Carcinogenesis* 30:1424–1432
 10. Bai Y, Edamatsu H, Maeda S, Saito H, Suzuki N, Satoh T, Kataoka T (2004) Crucial role of phospholipase cepsilon in chemical carcinogen-induced skin tumor development. *Cancer Res* 64:8808–8810
 11. Ou L, Guo Y, Luo C, Wu X, Zhao Y, Cai X (2010) RNA interference suppressing PLCE1 gene expression decreases invasive power of human bladder cancer T24 cell line. *Cancer Genet Cytogenet* 200:110–119
 12. Wang X, Zbou C, Qiu G, Fan J, Tang H, Peng Z (2008) Screening of new tumor suppressor genes in sporadic colorectal cancer patients. *Hepatogastroenterology* 55:2039–2044
 13. Bourguignon LY, Gilad E, Brightman A, Diedrich F, Singleton P (2006) Hyaluronan-CD44 interaction with leukemia-associated RhoGEF and epidermal growth factor receptor promotes Rho/Ras co-activation, phospholipase C epsilon-Ca²⁺-signaling, and cytoskeleton modification in head and neck squamous cell carcinoma cells. *J Biol Chem* 281:14026–14040
 14. Abnet CC, Freedman ND, Hu N, Wang Z, Yu K, Shu XO, Yuan JM, Zheng W, Dawsey SM, Dong LM, Lee MP, Ding T, Qiao YL, Gao YT, Koh WP, Xiang YB, Tang ZZ, Fan JH, Wang C, Wheeler W, Gail MH, Yeager M, Yuenger J, Hutchinson A, Jacobs KB, Giffen CA, Burdett L, Fraumeni JF Jr, Tucker MA, Chow WH, Goldstein AM, Chanock SJ, Taylor PR (2010) A shared susceptibility locus in PLCE1 at 10q23 for gastric adenocarcinoma and esophageal squamous cell carcinoma. *Nat Genet* 42:764–767
 15. Schaeffeler E, Zanger UM, Eichelbaum M, Asante-Poku S, Shin JG, Schwab M (2008) Highly multiplexed genotyping of thio-purine s-methyltransferase variants using MALD-TOF mass spectrometry: reliable genotyping in different ethnic groups. *Clin Chem* 54:1637–1647
 16. Kelley GG, Reks SE, Ondrako JM, Smrcka AV (2001) Phospholipase C(epsilon): a novel Ras effector. *EMBO J* 20:743–754
 17. Song C, Hu CD, Masago M, Kariyai K, Yamawaki-Kataoka Y, Shibatohe M, Wu D, Satoh T, Kataoka T (2001) Regulation of a novel human phospholipase C, PLCepsilon, through membrane targeting by Ras. *J Biol Chem* 276:2752–2757
 18. Downward J (2003) Targeting RAS signalling pathways in cancer therapy. *Nat Rev Cancer* 3:11–22
 19. Ma H, Wang LE, Liu Z, Sturgis EM, Wei Q (2011) 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. *BMC Cancer* 11:258
 20. Wang X, Zhou C, Qiu G, Yang Y, Yan D, Xing T, Fan J, Tang H, Peng Z (2011) Phospholipase C epsilon plays a suppressive role in incidence of colorectal cancer. *Med Oncol* 2:1051–1058
 21. Zhang H, Jin G, Li H, Ren C, Ding Y, Zhang Q, Deng B, Wang J, Hu Z, Xu Y, Shen H (2011) Genetic variants at 1q22 and 10q23 reproducibly associated with gastric cancer susceptibility in a Chinese population. *Carcinogenesis* 32:848–852