RESEARCH ARTICLE

PLCE1 Gene in Esophageal Cancer and Interaction with Environmental Factors

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Abstract

Objective: To study the PLCE1 gene rs2274223 polymorphism with regard to esophageal cancer and its interaction with diet, lifestyle, psychological and environmental factors in Southwest Shandong province. Materials and Methods: A case series study (case-case) was conducted. Questionnaire data were collected and 3 ml-5ml venous blood was drawn for DNA extraction among the qualified research subjects. PLCE1 gene polymorphism was detected after PCR amplification of DNA. SPSS 13.0 software was used for statistical analysis of the data. Results: The three genotypes A/A, A/G and G/G PLCE1 gene rs2274223 was 31, 16 and 4 cases, accounting for 60.8%, 31.4%, 0.08% respectively. The difference of three genotypes (AA/GA/GG) proportion between negative and positive family history of patients was statistically significant, χ^2=6.213, p=0.045. There was no statistically significant relationship between PLCE1 gene rs2274223 polymorphism and smoking, drinking, χ^2=0.119, p=0.998, and χ^2=1.727, p=0.786. There was no linkage of the three rs2274223 PLCE1 gene genotypes (AA/GA/GG) proportion with eating fried, pickled, hot, mildew, overnight, smoked, excitant food, eat speed, salt taste or not (p>0.05). or with living environment pollution and nine risk factors of occupational exposure (p>0.05). There was no statistically significant difference in TS scores between different genotype of rs2274223 PLCE1 gene. Conclusions: The PLCE1 rs2274223 polymorphism has a relationship with family history of esophageal cancer, but does not have any significant association with age, gender, smoking, alcohol drinking, food hygiene, eating habits, living around the environment and occupation in cases.

Keywords: Esophageal carcinoma - PLCE1 gene - case series study - gene polymorphism - interaction

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Introduction

Esophageal cancer is a kind of malignant tumor which occurred in esophageal epithelial tissue, also is one of the six common carcinomas in the world (Konda et al., 2008; Lin et al., 2009). Globally, Almost 400 thousand people were diagnosed with esophageal cancer every year, more than 80% in developing countries (Ferlay et al., 2010). Both the crude morbidity and mortality of esophageal cancer in China were highest in the world, the incidence of the world standard rate was in sixth place (male sixth, female tenth), the death rate of the world standard rate was twelfth (male eleventh, female sixteenth) (Zhang et al., 2012).

The etiology of esophageal cancer is complex, and the exact pathogenesis of esophageal cancer occurrence remains unclear, despite lack of vitamins in the diet, eating pickled vegetables, food contain nitrite or fungal contamination of food, smoking, alcohol consumption were the main risk factors of esophageal cancer in some reports (Fan et al., 2008; Song et al., 2009). In recent years, studies have shown that, PLCE1 was associated with the occurrence of a variety of human cancer, especially that the relationship with gastrointestinal tumorigenesis has become a hotspot. PLCE1 gene polymorphism rs2274223 is currently one of the most studied loci (Abnet et al., 2010; Hao et al., 2010; Cui et al., 2013), which is a independent risk factor of squamous cell carcinoma of the esophagus and stomach cancer. Rs2274223 PLCE1 gene loci locates in 26th exons, which makes amino acids changing from histidine to arginine and makes PLCE1 mRNA and the expression level of PLCE1 protein up-regulation in the organizition of esophageal cancer.

Our study will start from etiology pathogenesis of esophageal cancer and explore the interaction among many factors in the onset of esophageal cancer. Therefore, our study will discuss PLCE1 gene expression of patients with esophageal cancer of west-south in Shandong Province. The simple case research method will be used to explore the interaction in esophageal PLCE1 gene

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polymorphism and diet, lifestyle, psychological and environment factors. To further understand the biomarkers - PLCE1 gene polymorphism on cancer risk prediction and clinical pathologic correlation value and significance of exploring a variety of pathogenesis, so as to reveal the relation among many etiology pathogenesis to provide important theoretical basis for primary prevention, early diagnosis and prognosis assessment of the esophageal cancer.

Materials and Methods

Research subjects

New cases of esophageal cancer without radiotherapy and chemotherapy were continuous collected as the research subjects in March 2014 to June 2014. The participants were exactly diagnosed as cases of esophageal cancer by histopathological examination and extration of cancer about other systems from the Department of Thoracic Surgery in First People's Hospital of Jining city and the Affiliated Hospital of Jining Medical University. All the research subjects were the Han race and had no blood relationship, they were geographical mainly from 12 counties and the region of Jining Shandong province and had lived there for more than 20 years. The sample was well representative of the source population. All the research subjects signed the informed consent before the investigation and specimens being collected. 5ml whole blood was gathered of each case, EDTA anticoagulant, -20°C refrigerator stored.

Methods

A case series study (case-case study) method was used. Questionnaire investigation was done and 3ml-5ml venous blood was drawn among the qualified research subjects. Then PLCE1 gene polymorphism was detected. To study the association of esophageal cancer and PLCE1 gene polymorphism about residents of southwest of Shandong province through data analysis, so as to investigate interaction of esophageal PLCE1 gene polymorphism and diet, lifestyle, psychological and environmental factors.

The questionnaire investigation

The investigation was carried by self-designed questionnaire. The following five aspects were surveyed to the patients and their families: 1. Demographic characteristics: gender, age, marital status, educational level, occupation, family history of disease and etc. 2. Diet, lifestyle and habits: including food factors and eating habits. Food factors included staple food and local habitual food, such as cottonseed oil, and dry food, spicy food, fried food, etc. Eating habits included temperature of food, food preferences, feeding speed, dine regularity, drinking habits, smoking, drinking, etc. 3. Living environments: such as type of drinking water, usage of pesticides and fertilizers on the surrounding, treatment of sewage and garbage pollution, etc. 4. Psychological factors: the depression, anxiety scale were used to analysis the psychological factors influencing the esophageal cancer occurs.

Specimen collection and analysis

i) The extraction of DNA from the whole blood : using DNA extraction kit, according to the kit instructions, DNA were totally extracted, then, using ultraviolet spectrophotometer detection to extract the OD value of DNA, the extraction of DNA were repackaged, saved in -20°C refrigerator, set aside. *ii)* PCR amplification: primer sequence synthesis by Shanghai Sangon biological company. Rs2274223 loci: 5'-TGTCTCCCTCACCCTAGATTGT-3'(upstream) and 5'- AAATTCTGGCATCCATCACCT-3' (downstream), product size was 210 bp. PCR amplification was in 50ul reaction system. Reaction conditions as follows: 94°C modified 2 minutes beforehand; 94°C modified 30 seconds, annealing 30 seconds to 60°C, 72°C extension for 30 seconds, a total of 30 cycles; 72°C extension for 2 minutes. *iii)* Electrophoresis of PCR products: 5ul PCR products were got out, then were added to the pre-made 1% agarose gel point sample hole, voltage 150 v, the termination of electrophoresis was within 20 minutes, gel was included in the gel analyzer pictures for future reference. *iv)*. Determination of genotypes: The PCR products were sent to Shanghai Sangon biological company for sequencing. The genes after sequencing compared with the NCBI databases, proved to be the required purpose fragments, sequence map viewed with chromas software, rs2274223 genotype was AA, AG, GG, respectively.

Statistical analysis

After all the questionnaire being collected, the data were put in EpiData 3.0 software. The statistical software SPSS 13.0 was used for statistical analysis of data. Count data were used χ^2 analysis, AA group and AG/GG psychosocial stress factors were conducted independent sample test. α=0.05 was the significant level, p<0.05 meant the difference was statistically significant.

Results

The experimental results

According to the PCR amplification product electrophoresis results that 51 of 52 cases' DNA samples of PCR products were qualified, eliminate 17 samples, (Figure 1).

The sequencing of rs2274223 PLCEI gene loci mapping result was shown in figure 2, the number of three genotypes A/A, A/G and G/G was 31, 16 and 4, accounted for 60.8%, 31.4%, 0.08%, respectively.

Interactions of rs2274223 PLCE1 gene loci polymorphism with environmental factors

i) Relationship of rs2274223 PLCE1 gene loci polymorphism to age, gender, and family history. The average age of 51 cases who were genotyped was

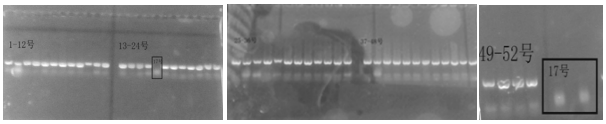

Figure 1. DNA Samples from the PCR Amplification Product Electrophoresis

(64.6+8.1). The youngest age was 46 years old, the oldest was 83 years old. Comparison by groups of age <65 years old and ≥65 year old, χ^2= 1.906, p=0.386, so in the α= 0.05 significance level, rs2274223 locus polymorphism and age could not be considered statistically significant link. The male in 52 patients with esophageal cancer were 39 cases, accounting for 74.5%, female 13 cases, accounted for 25.5%, male to female ratio was 3:1. There was no significant difference of three genotypes (AA/GA/GG) proportion between male and female patients of 51 cases, χ^2=0.588, p=0.745.

The difference of three genotypes (AA/GA/GG) proportion between negative and positive family history of patients was statistically significant, χ^2=6.213, p=0.045. The proportion of AA genotype and AG was 59.1% and 22.7% among patients with positive family history, was less than patients with a negative family history, the proportion of AA genotype and AG was 62.1% and 57.8%. But the proportion of GG genotype (18.2%) with the positive family history was significantly higher than patients with a family history of negative (0%), (Table 1). *ii)* The relationship between PLCE1 gene rs2274223 polymorphism and smoking, drinking. About 50% of them were smokers, non-smokers accounted for 30.8%, ex-smoker was 19.2%. Drinkers accounted for 46.2%, non drinkers accounted for 36.5%, of those who had quit accounted for 17.3%. The relationship between

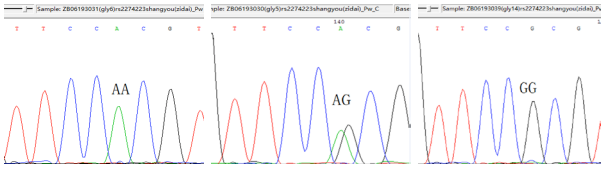

Figure 2. Sequencing Results Map rs2274223 PLCE1 gene Loci

Table 1. Relationship between rs2274223 PLCE1 gene Loci Polymorphism's and Age, Sex and Family History

Variables	Gene type			χ^2	P
	AA	AG	GG		
Age (years old)					
<65	12	7	3	1.906	0.386
≥65	19	9	1		
Gender					
Male	22	13	3	0.588	0.745
Female	9	3	1		
Family history					
Yes	13	5	4	6.213	0.045
No	18	11	0		

Table 2. The Relationship between PLCE1 gene rs2274223 Polymorphism and Smoking, Drinking

Variables	Classification	Genotype			χ^2	P
		AA	AG	GG		
Smoking						
	Yes	16	8	2	0.119	0.998
	No	9	5	1		
	Quit smoking	6	3	1		
Drinking	Yes	14	9	1	1.727	0.786
	No	12	4	2		
	Temperance	5	3	1		

Table 3. Relationship between the PLCE1 gene rs2274223 Polymorphism and Food Health, Diet

Variables	Classification	Gene type			χ^2	P
		AA	AG	GG		
Fresh vegetables	Don't eat / Occasionally	11	7	1	0.587	0.746
	Regularly	20	9	3		
Fresh vegetables	Don't eat / Occasionally	20	13	4	7.267	0.122
	Regularly	11	3	0		
Meat egg milk	Don't eat / Occasionally	16	9	3	1.978	0.740
	Regularly	16	7	1		
Beans	Don't eat / Occasionally	14	8	1	7.516	0.111
	Regularly	17	8	3		
Garlic	Don't eat / Occasionally	22	13	3	3.915	0.418
	Regularly	9	3	1		
Pickled food	Don't eat	2	2	0	4.076	0.396
	Occasionally/Regularly	29	14	4		
Fried food	Don't eat	3	2	1	1.779	0.776
	Occasionally/Regularly	28	13	3		
Hot food	Don't eat	5	3	1	0.843	0.933
	Occasionally/Regularly	26	13	3		
Mouldy food	Don't eat	22	11	3	2.279	0.685
	Occasionally/Regularly	9	5	1		
Overnight food	Don't eat	5	2	0	1.197	0.879
	Occasionally/Regularly	26	14	4		
Smoked food	Don't eat	21	8	2	1.605	0.448
	Occasionally/Regularly	10	8	2		
Excitant food	Don't eat	12	4	0	7.372	0.117
	Occasionally/Regularly	19	12	4		
Eat speed	Slow/Moderate	22	9	3	3.029	0.553
	Fast	31	16	1		
Salt taste	Pale/Medium	20	9	2	1.060	0.900
	Emphase on taste	11	7	2		

Table 4. Relationship between PLCE1 gene rs2274223 Polymorphism and the Environment, Occupation

Variables	Classification	Gene type			χ^2	P
		AA	AG	GG		
Environment pollution	Yes	15	6	3	1.862	0.394
	No	16	10	1		
Asbestos industry	Yes	0	0	0	——	——
	No	31	16	4		
Pesticides and herbicides	Yes	17	13	3	3.425	0.180
	No	14	3	1		
The paint industry	Yes	1	2	0	1.911	0.385
	No	30	14	4		
Printing business	Yes	0	0	0	——	——
	No	31	16	4		
Rubber industry	Yes	0	0	0	——	——
	No	31	16	4		
Dye industry	Yes	0	0	0	——	——
	No	31	16	4		
Automotive industry	Yes	1	0	0	0.698	0.720
	No	30	16	4		
Metal industry	Yes	1	1	0	0.433	0.805
	No	30	15	4		
Decoration industry	Yes	2	0	0	1.343	0.511
	No	29	16	4		

Table 5. Comparison of Psychosocial Stress Score between AA group and AG/GG Group

Emotional factors	Gene type		t	P
	AA	AG/GG		
L	1.97±1.91	2.57±2.23	-1.047	0.300
NE	2.55±2.85	2.43±2.16	0.163	0.871
PE	2.35±1.72	2.71±2.15	-0.668	0.507
NC	3.22±2.50	3.24±1.92	0.131	0.896
PC	4.48±2.76	3.43±2.06	1.493	0.142
TS	36.35±24.80	37.48±18.43	-0.177	0.861

PLCE1 gene rs2274223 polymorphism and smoking, drinking had no statistical significance (Table 2). *iii)* The relationship between rs2274223 PLCE1 gene loci polymorphism and the food health, diet. 14 kinds of related factors of food hygiene and eating habits of the incidence of esophageal cancer were surveyed. The analysis found that there was no statistically significant difference of three rs2274223 PLCE1 gene genotypes (AA/GA/GG) proportion between the groups of eating fried, pickled, hot, mildew, overnight, smoked, excitant food, eat speed, salt taste or not. (Table 3). *iv)* Relationship between Rs2274223 PLCE1 gene loci polymorphism and career, environment. 24 cases of 51 patients who lived with environment pollution, accounted for 47.1%. The relationship between Rs2274223 PLCE1 gene loci polymorphism and whether the living environment pollution or not was no statistical significance (χ^2=1.682, p=0.394). There were 9 kinds risk factors of occupational exposure associated with the incidence of esophageal cancer which were asked in the questionnaire. There were 33 cases of occupational exposure to pesticides or herbicides, accounted for 63.5%. 4 cases had been exposed to paint industry occupational exposure, accounted for 7.7% of the total number of patients. 2 cases had been exposed to car industry, accounted for 3.8% of the total number of patients, so as to the number and proportion of metal and decoration industry exposure. 1 case had been exposed to lead rubber industry, accounted for 1.9% of the total number of patients. There were no asbestos industry and printing of professional contacts. There was no statistically significant difference of three rs2274223 PLCE1 gene genotypes (AA/GA/GG) proportion between the groups of nine occupational exposure to risk factors of or not. (Table 4). *v)* Relationship between Rs2274223 PLCE1 gene loci polymorphism and psychosocial stress

Psychosocial stress scale contained 44 entries. Stress factors were divided into 5 parts include life events (L), negative emotion experience (NE), positive emotional experience (PE), negative coping NC and positive coping with (PC). Total stress scores was TS=15+2L+3NE-PE+5NC-PC. Independent samples T test was conducted in each part. There was no statistically significant difference in TS and each of the scores about five parts between different genotype of rs2274223 PLCE1 gene. So we cannot think rs2274223 PLCE1 gene loci polymorphism was associated with psychosocial stress factors. (Table 5).

Discussion

Etiology process of esophageal cancer was complicated, and there was no definite conclusion about the cause of the esophageal cancer now. But the universal opinion considered that there were a combination of many factors in the development of esophageal cancer(Gbadegesin et al., 2009; Bunney et al., 2010). Phospholipase CE1 (PLCE1) was a recently discovered isozyme of phospholipase C family, from cell membrane to the nucleus in mediating cell signal transmission in the process which played an important role, regulated cell growth, differentiation and related gene expression (Ikuta et al., 2008; Li et al., 2009; Hu et al., 2010). Rs2274223 loci was in 26th exon of PLCE1 gene, makes amino acids change from the histidine to arginine. Rs2274223 PLCE1 gene polymorphism loci was currently one of the

most studied loci, which was an independent risk factor for esophageal cancer(Wang et al., 2010; Guo et al., 2014). In addition to genetic factors, smoking, drinking alcohol, food hygiene, eating habits, living environment around and occupational factors may related with the incidence of esophageal cancer. However, compared with people who have a family history of esophageal cancer, the proportion of people prone to esophageal cancer was minimal under the same environmental factors. It suggested that an individual's genetic predisposition to the environment factors' exposure associated with esophageal cancer. And individual genetic susceptibility under the condition of environmental exposure factors have synergy to esophageal cancer which result in higher incidence of esophageal cancer. The interaction of the genetic susceptibility and environmental factors play an important role in the process of esophageal cancer, and is under the influence that environment factors make changes caused by multiple genes.

Our research showed that the average age of the patients with esophageal cancer was (64.6+8.1), there was no significant differences of PLCE1 gene polymorphism between different ages. Gender and esophageal cancer gene polymorphism with no correlation, but there were much more male cases than female ones. This survey showed that the ratio of male cases and female cases was 3:1. Relevant information speculated that testosterone might be risk factors of esophageal cancer among men and estrogen might be protective among women. A study showed rs2274223 PLCE1 gene genetic polymorphisms in esophageal squamous carcinoma (HCC), but with no obvious interaction between smoking, alcohol consumption, body mass index (Song et al., 2012). This study also did not find interaction of esophageal rs2274223 PLCE1 gene loci polymorphism with smoking, drinking alcohol, food hygiene, eating habits, the living environment and occupational exposure. Our study found 43.1% patients had a positive family history of esophageal cancer, suggested that genetic susceptibility of esophageal cancer patients with positive family history was strong, and there were more people susceptible to esophageal cancer under the same environment. So the most important is screening and prevention among those who have family history of esophageal cancer.

Our study suggested the proportion of GG genotype with the positive family history was significantly higher than patients with a family history of negative, so that loci G allele of rs2274223 PLCE1 gene in patients with esophageal cancer may be thought increasing the risk of carcinoma. Rs2274223 PLCE1 gene loci polymorphism can be an effective genetic marker used as an assessment of the risk of esophageal cancer. But the sample size of this research was small, only 51 cases, may affect the stability of the multifactor interaction analysis results. Next we will continue to expand the sample size to provide important theoretical basis of PLCE1 gene polymorphism on cancer risk prediction and interaction with other traditional factors, and set up genetic carcinogenic testing model that can joint PLCE1 gene and other traditional factors.

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