

Original Article

Relationship between *hWAPL* polymorphisms and cervical cancer susceptibility

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Abstract: Purpose: To analyze the correlation of the polymorphisms of human wing-apart like (*hWAPL*) gene (rs7083506 and rs11202058) with the susceptibility to cervical cancer. Besides, the relationship of haplotypes between the polymorphisms with cervical cancer susceptibility was analyzed. Methods: Taqman probe genotyping method was adopted to detect the genotype distribution of *hWAPL* rs7083506 and rs11202058 polymorphisms in 117 cervical cancer patients and 128 healthy controls. Linkage disequilibrium and haplotypes were analyzed by Haploview software. χ^2 test was utilized to analyze the differences of genotype, allele and haplotype frequencies between the case and control groups. Results: Correlation analysis of *hWAPL* rs7083506 and rs11202058 polymorphisms with cervical cancer susceptibility was based on the five genetic models. TT genotype of rs7083506 increased the susceptibility of cervical cancer in TT vs. CC model and TT vs. CT+TT model (OR=2.249, 95% CI=1.018-4.970; OR=2.287, 95% CI=1.069-4.896). For rs11202058, the A allele increased the cervical cancer susceptibility (A vs. G, OR=1.502, 95% CI=1.005-2.245). No significant correlation was observed between rs11202058 genotypes and cervical cancer susceptibility. We performed the haplotype analysis between the two polymorphisms, and found that T-A haplotype significantly correlated with cervical cancer, the susceptibility of cervical cancer increased to 1.78 times. Conclusions: Rs7083506 and rs11202058 polymorphisms of *hWAPL* and their haplotype T-A were associated with cervical cancer.

Keywords: *hWAPL*, cervical cancer, polymorphisms, haplotype

Introduction

Cervical cancer is one of the malignant tumors that cause serious damage to the health of women around the world. What's more, the morbidity of cervical cancer is increasing and the average onset age of the disease is getting lower [1, 2]. The occurrence and development of cervical cancer is a multi-step process which is influenced by multiple factors including genetic and environmental factors [3-7]. Numerous current studies have reported that genetic factors are the main cause of cervical cancer. There exist a close relationship between genetic polymorphisms and cervical cancer susceptibility [8-11].

Wing-apart Like (*WAPL*) gene is a new gene which was firstly found in fruit flies [12, 13]. Human *WAPL* (*hWAPL*) gene is the homologous

sequence of *WAPL*. Human *WAPL* gene has a length of 30793 bp and is located on chromosome 10q23.2. Specific functional mechanism of the *hWAPL* gene is not clear yet. But, as we all know *hWAPL* gene encodes a kind of aggregated anchored protein that can timely disassemble the polymerization of chromosome arms at the early stage of mitosis [14]. A lot of studies have proved that *hWAPL* gene is highly expressed in cervical cancer patients, and the expression level of *hWAPL* gene has a positive correlation with the lesion degree of cervix [15-18]. At present, studies on *hWAPL* are at a premature stage. The mechanism of *hWAPL* gene in cervical cancer still unknown, and more studies are needed to further reveal the mechanism of cervical cancer induced by *hWAPL* gene. There is scarcely research focusing on the correlation between *hWAPL* gene polymorphisms and cervical cancer.

hWAPL polymorphisms and cervical cancer

Table 1. Genotype and allele distributions of rs7083506 and rs11202058

Genotype/Allele	Case n=117 (%)	Control n=128 (%)	HWE
rs7083506			0.622
CC	43 (36.75)	60 (46.88)	
CT	51 (43.59)	57 (44.53)	
TT	23 (19.66)	11 (8.59)	
C	137 (58.55)	177 (69.14)	
T	97 (41.45)	79 (30.86)	
rs11202058			0.106
GG	63 (53.85)	85 (66.74)	
GA	38 (32.48)	35 (27.3)	
AA	16 (13.67)	8 (6.3)	
G	164 (70.09)	205 (80.1)	
A	70 (29.91)	51 (19.9)	

In current study, we selected two single nucleotide polymorphisms (SNPs) of *hWAPL* gene, namely rs7083506 and rs11202058, to discuss their relationship with the cervical cancer risk. Additionally, we analyzed the correlation between haplotypes of the SNPs with cervical cancer susceptibility.

Materials and methods

Research objects

In our study, we adopted a case-control design. All of the participants were unrelated Chinese Han population. 117 cervical cancer patients were selected from Shenzhen Guangming New District Center Hospital during the period from January 2010 to December 2014. 128 healthy individuals were recruited from the physical examination center of the same hospital during the same period. The age of cases ranged from 24 to 65 years old, while that of controls from 26 to 69. The average ages of the case and control groups were respectively 49 and 51 years old. Cervical cancer patients had been diagnosed by routine pathological examinations and did not undergo radiotherapy or chemotherapy before blood collection. Cytological examination and biopsy under colposcope of the cervix revealed that the controls do not have cervical cancer or precursor lesions thereof. Written informed consent was signed by all the participants. This study was approved by the ethics committee of Shenzhen Guangming New District Center Hospital. Sample collection

was conducted following the ethnical principles of National Human Genome Research Institute.

Sample preparation

5 ml fasting venous blood was collected from each participant, and then we extracted the genomic DNA with TIANamp blood DNA kit (Tiangen, China).

Genotyping of *hWAPL* rs7083506 and rs11202058 polymorphisms

Rs7083506 and rs11202058 polymorphisms of *hWAPL* gene were genotyped with Taqman probe genotyping method using Roche Light-Cycler 480 real-time fluorescence quantitative PCR instrument. *GAPDH* was used as endogenous control. Primers and probes of the two SNPs and *GAPDH* were designed and synthesized by Applied Biosystems Company. PCR reaction volume was 20 μ l, and PCR reaction conditions were presented as follows: 10 minutes initial denaturation at 95°C; then 40 cycles of 15 seconds denaturation at 92°C, 1minute annealing at 60°C, and 40 seconds extension at 72°C; and at last 5 minutes final extension at 72°C.

Statistical analysis

Hardy Weinberg Equilibrium (HWE) test was used to inspect the representativeness of the genotype frequencies. χ^2 test was adopted to compare the discrepancies of genotype and allele distribution frequencies of the two SNPs between case and control groups. Linkage disequilibrium and haplotype analyses of the two SNPs were assessed by Haploview software. Statistical calculation was performed by SPSS software 18.0. Statistically significant level of differences was $P < 0.05$.

Results

Correlation analysis of the two SNPs and cervical cancer

Genotype distributions of *hWAPL* rs7083506 and rs11202058 polymorphisms in the control group were in accordance with HWE (**Table 1**). The correlation between the SNPs and cervical cancer were shown in **Table 2**. For rs7083506, in TT vs. CC model and TT vs. CT+TT model the TT genotype were significantly increased the

Table 2. Correlation analysis of rs7083506 and rs11202058 with cervical cancer

Model	rs7083506		rs11202058	
	P value	OR (95% CI)	P value	OR (95% CI)
11 vs.22	0.042	2.249 (1.018-4.970)	0.057	2.354 (0.957-5.792)
11+12 vs. 22	0.409	1.191 (0.787-1.801)	0.187	1.374 (0.856-2.204)
11 vs. 12+22	0.030	2.287 (1.069-4.896)	0.077	2.188 (0.903-5.301)
12 vs. 22	0.731	1.085 (0.681-1.729)	0.346	1.290 (0.759-2.192)
Allele 1 vs. Allele 2	0.094	1.343 (0.950-1.898)	0.047	1.502 (1.005-2.245)

Notes: 1, variant allele; 2, ancestral allele.

Table 3. Linkage disequilibrium and haplotype analyses of alleles in rs7083506 and rs11202058 in cervical cancer

Locus 1-Locus 2	Case 2n=234 (%)	Control 2n=256 (%)	χ^2	P value	OR (95% CI)
C-G	137 (58.55)	177 (69.14)	-	-	1.00
T-A	69 (29.49)	50 (19.53)	7.127	0.008	1.783 (1.163-2.732)
T-G	28 (11.96)	29 (11.33)	0.589	0.443	1.247 (0.709-2.195)

Notes: Locus 1, rs7083506; Locus 2, rs11202058.

susceptibility of cervical cancer ($P=0.042$, $P=0.030$). No significant correlation existed in other models. An allele of rs11202058 SNP was obviously correlated with cervical cancer susceptibility (A vs. G, $P=0.047$). There were no significant associations between the genotypes of rs11202058 and the susceptibility of cervical cancer.

Haplotype analysis of hWAPL rs7083506 and rs11202058

Strong linkage disequilibrium existed between rs7083506 and rs11202058 ($D'=1$, $r^2=0.734$). So we performed the haplotype analysis between rs7082506 and rs11202058. Besides, the correlations between the haplotypes and cervical cancer were inspected and shown in **Table 3**. We found that discrepancies of the distribution frequency of T-A haplotype in two groups were statistically significant ($P=0.008$). The result indicated that T-A haplotype had positive correlation with cervical cancer. However, T-G haplotype had no significant correlation with cervical cancer.

Discussion

Cervical cancer is one of the most common malignant tumors of female genital tract. Cervical cancer has the highest incidence and mortality among all gynecological tumors. Some

studies concerning the pathogenesis of cervical cancer have demonstrated that multiple factors like premature first sexual life, sex life disorders, preterm, dense and productive births, bad economic status and etc. are related to cervical cancer incidence. In 1980, Reid et al. found human papillomavirus (HPV) in cervical biopsy samples [19]. Afterwards, a lot of studies showed that HPV was significantly correlate with cervical cancer [20, 21]. At present, HPV is generally regarded as the major patho-

genic factor of cervical cancer, and research have revealed that cervical HPV infection is intimately tied to the progress of cervical cancer lesions [22-24]. However, HPV infection is only a necessary but not sufficient condition for cervical cancer occurrence. With the development of molecular genetics [25], people gradually realize that the occurrence of cervical cancer is the result of interactions between environmental factors and genetic factors [26, 27]. Different genetic susceptibility of disease is determined by gene polymorphisms, which is also the case for cervical cancer. Genetic susceptibility is one of the key factors that affect the occurrence of cervical cancer after HPV infection in the host [28-30].

Oikawa et al. demonstrated that hWAPL is highly expressed in cervical cancer [15]. hWAPL protein is a kind of agglutination-protein binding protein which can enable the agglutination protein to separate from the chromosome arm at the beginning of cell division. The reason of the specific and high expression of hWAPL gene in cervical cancer patients is not clear yet. Latest study discover that hWAPL is specifically and highly expressed in the cells infected by HPV, and thus is closely related to the risk of cervical cancer [31]. Some related studies have also proved the existence of a certain relationship of high-risk HPV infection with hWAPL

expression [17, 18, 32]. So we can believe that there is a certain association between *hWAPL* gene and cervical cancer susceptibility. Nevertheless, we have seen few reports about the linkage between *hWAPL* gene polymorphisms and the incidence of cervical cancer. Therefore, a case-control study was carried out to analyze the relevance between *hWAPL* polymorphisms (rs7083506 and rs11202058) and cervical cancer incidence.

Calculation results of our study indicated that TT genotype and T allele of *hWAPL* rs7083506 were apparently higher in case group than that in control group. There were significant distribution differences of AA genotype and A allele of rs11202058 between case and control groups. Herein, we analyzed the correlation of the two SNPs with cervical cancer based on five genetic model. Then we found that rs7083506 TT genotype could increase the susceptibility of cervical cancer in TT vs. CC model and TT vs. CT+CC model with 2.249 and 2.287 fold, respectively. But the T allele of rs7083506 had no significant association with cervical cancer. Contrast with rs7083506, no significant associations existed between all of the rs11202058 genotypes and cervical cancer. A allele of rs11202058 increased the susceptibility of cervical cancer with 1.502 fold (A vs. G model). We also assessed the linkage disequilibrium and haplotypes of *hWAPL* rs7083506 and rs11202058 polymorphisms in cervical cancer. To our knowledge, this is the first study focused on the correlation between haplotypes of rs7083506 and rs11202058 with cervical cancer susceptibility. In current study, the results showed that T-A haplotype obviously increased the cervical cancer susceptibility, with the odd ratio of 1.247. There was no significant association between T-G haplotype and cervical cancer.

Combined the aforementioned results, we suggested that rs7083506 and rs11202058 of *hWAPL* might act as the susceptible polymorphisms of cervical cancer. However, the association between *hWAPL* polymorphisms and clinical pathological indicators and prognosis of cervical cancer need to be further researched.

Disclosure of conflict of interest

None.

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hWAPL polymorphisms and cervical cancer

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