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Original Article

Cyclin D1 G870A polymorphism and glioma risk in a Chinese population

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Abstract: Background: A number of studies have suggested that the Cyclin D1 (CCND1) G870A polymorphism was associated with susceptibility to various cancers. In the present study, we aimed to investigate the association between CCND1 G870A polymorphism and the risk of glioma in a Chinese population. Materials and methods: CCND1 genotyping was determined by the PCR-RFLP method. The χ^2 test was used to assess for any deviation of the genotype frequencies from Hardy-Weinberg equilibrium and to compare the genotype distributions among glioma patients and healthy control subjects. We calculated the odds ratios (ORs) and 95% confidence intervals (95% CIs) by using unconditional logistic regression. Results: The A allele frequency was higher in cases than that in controls (49.40% vs. 36.39%), and this difference was statistically significant (P = 0.001). Using the G allele as the reference allele, the subjects carrying the A allele had 3.926-fold increase in the risk of glioma (95% CI, 2.172-7.889), and *p*-value was significant (P = 0.007). Compared to individuals with the GG genotype, individuals with the AA genotype exhibited significantly increased glioma risk (OR = 3.661, 95% CI: 1.658-6.287, P = 0.01). Conclusion: Our results suggest that the CCND1 G870A polymorphism may contribute to the susceptibility to glioma in Chinese population.

Keywords: Glioma, risk, cyclin D1 gene, polymorphism, case-control study

Introduction

Glioma is the most common tumor of the central nervous system in adults, accounting for > 70% of all types of brain tumor [1]. The mechanisms of carcinogenesis for glioma are still not fully understood. Prolonged exposure to exogenous carcinogenic substances, such as ionizing radiation, is recognized as an important stimulus to the initiation and formation of glioma [2-4]. The impact of genetics has received widespread attention in recent years [5, 6].

Cyclin D1 is encoded by the CCND1 gene, located on chromosome 11q13. It is an important regulator protein for the G1-S cell cycle phase transition and has an impact on the regulation of cell proliferation and differentiation. Germline genetic variability is common in cyclin D1, for example, single nucleotide polymorphisms (SNPs). These common SNPs may alter the function and activity of cyclin D1 gene, consequently causing differences in individual susceptibility to cancer progression. In exon 4 of cyclin D1, there lies a silent G to A substitution at nt870 (rs603965) related to the increase of cyclinD1 expression [7]. A number of studies have suggested that the CCND1 G870A polymorphism is associated with susceptibility to various cancers, including esophageal adenocarcinoma, breast cancer, lung cancer, endometrial cancer, prostate cancer, bladder cancer, hepatocellular carcinoma, colorectal cancer, and cervical cancer [8-14]. Previously, a few studies have investigated the association between CCND1 G870A polymorphism and the risk of glioma, however, the conclusions were not consistent [15-17]. In the present study, we aimed to investigate the association between CCND1 G870A polymorphism and the risk of glioma in a Chinese population.

Materials and methods

Study Population

This study was approved by the ethics committee of The Third Xiangya Hospital of Central

Table 1. Characteristics of the 167 glioma cases and 180 healthy control subjects

Characteristics	Cases (n = 167)		Controls (n = 180)		*P*-value
	n	%	n	%	
Gender					
Male	101	60.48	103	57.22	0.586
Female	66	39.52	77	42.78	
Age (years)					
≤ 60	87	52.10	82	45.56	0.238
> 60	80	47.90	98	54.44	
Smoking					
Ever	121	72.46	139	77.22	0.323
Never	46	27.54	41	22.78	
Alcohol drinking					
Ever	143	85.63	166	92.22	0.059
Never	24	14.37	14	7.78	

Table 2. Genotype frequencies of CCND1 G870A polymorphism between glioma cases and healthy controls

	Cases (n = 167)		Controls (n = 180)		*P*-value
	n	%	n	%	
Genotype					
GG	61	36.53	82	45.56	0.002
AG	47	28.14	65	36.11	
AA	59	35.33	33	18.33	
Allele					
G	169	50.60	229	63.61	0.001
A	165	49.40	131	36.39	

South University. All the participants signed the inform consent before this study. We performed a hospital-based case-control study. A total of 167 patients with glioma and 180 healthy controls were qualified for this study. All samples were taken at the Department of Neurosurgery, The Third Xiangya Hospital of Central South University, between March 2010 and June 2014. The healthy, unrelated and cancer-free subjects, who visited hospital for a routine health checkup, were recruited for the study as controls. The samples were collected from glioma patients before any chemotherapeutic or radiation therapy treatment had been started. All subjects in this study were genetically from Hunan province and the adjacent area in China, with a Han Chinese ethnic background.

Genotyping assays

A 5 ml sample of peripheral blood was taken in the operation theatre preoperatively in all patients. Genomic DNA (germ line DNA) was extracted from peripheral blood leukocytes and purified according to established protocols using the QIAamp Blood Tissue Kit. Primers used for CCND1 A870G were: forward 5'-GTG AAG TTC ATT TCC AAT CCG C-3', and reverse 5'-GGG ACA TCA CCC TCA CTT AC-3'. Polymerase chain reaction (PCR) cycling conditions were: one cycle at 94°C for 5 min; 35 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s, and a final extension at 72°C for 10 min. The PCR products were digested with 5 U MspI enzyme (MBI Fermentas, Lithuania) at 37°C for overnight and electrophoresed on 4% agarose gel. The allele types were determined as follows: two fragments of 175 and 37 bp for AA genotype, three fragments of 141, 37 and 34 bp for GG genotype, and four fragments of 175, 141, 37 and 34 bp for AG genotype.

Statistical analyses

The χ^2 test was used to assess for any deviation of the genotype frequencies from Hardy-Weinberg equilibrium and to compare the genotype distributions among glioma patients and healthy control subjects. We calculated the odds ratios (ORs) and 95% confidence intervals (95% CIs) by using unconditional logistic regression. SPSS 18.0 software was utilized to perform the data analysis. Statistical significance was established at $P < 0.05$.

Results

Characteristics of the glioma cases and healthy control subjects

The clinical characteristics of the 167 patients with glioma and the 180 healthy control subjects are presented in **Table 1**. We found that there were no significant differences identified in the distribution of gender (P = 0.586), age (P = 0.238), smoking status (P = 0.323), alcohol consumption status (P = 0.059) between the two groups (shown in **Table 1**).

Genotype frequencies of CCND1 G870A polymorphism between glioma cases and healthy controls

The distributions of the CCND1 genotypes were in Hardy-Weinberg equilibrium in the glioma

Table 3. The association of CCND1 G870A polymorphism with glioma risk

	Patients	Controls	OR (95% CI)[1]	*P* value
General genotype				
GG	61	82	1.00 (Reference)	
AG	47	65	2.972 (0.782-3.874)	0.09
AA	59	33	3.661 (1.658-6.287)	0.01
Dominant genotype				
GG	61	82	1.00 (Reference)	
AG + AA	106	98	3.076 (0.899-5.919)	0.08
Recessive genotype				
GG + AG	108	147	1.00 (Reference)	
AA	59	33	2.168 (0.825-5.917)	0.11
Allele frequency				
G	169	229	1.00 (Reference)	
A	165	131	3.926 (2.172-7.889)	0.007

[1]Adjusted for sex, age, smoking status, and drinking status.

patients (P > 0.05) and the controls (P > 0.05). Distributions of the CCND1 genotype and allele frequency between cases and controls were analyzed using Pearson's χ^2 test and shown in **Table 2**. The A allele frequency was higher in cases than that in controls (49.40% vs. 36.39%), and this difference was statistically significant (P = 0.001). The frequencies of the GG, AG, and AA genotypes were 36.53%, 28.14%, and 35.33% in the glioma cases, respectively, and 45.56%, 36.11%, and 18.33% in the controls respectively, and the difference was statistically significant (P = 0.002, shown in **Table 2**).

The association of CCND1 G870A polymorphism with glioma risk

To evaluate the risk of glioma according to the CCND1 G870A allele and genotype, logistic regression analysis was conducted with adjustment for age, sex, smoking status and alcohol use. Using the G allele as the reference allele, the subjects carrying the A allele had 3.926-fold increase in the risk of glioma (95% CI, 2.172-7.889), and *p*-value was significant (P = 0.007). Compared to individuals with the GG genotype, individuals with the AA genotype exhibited significantly increased glioma risk (OR = 3.661, 95% CI: 1.658-6.287, P = 0.01). Under the dominant model of inheritance, the AG + AA genotype was not significantly associated with increased risk for glioma (OR = 3.076, 95% CI = 0.899-5.919; P = 0.08). Moreover, under the recessive model of inheritance, the AA genotype was not significantly associated with increased risk for glioma (OR = 2.168, 95% CI = 0.825-5.917; P = 0.11, shown in **Table 3**).

Discussion

The estimated five-year survival rate is 60% and 74% for biopsy, and watchful waiting and early resection in low-grade gliomas, respectively [5, 18]. Gliomas are an enigmatic and heterogeneous disease, the exact etiology of which remains unclear. Certain factors are found to affect an individual's glioma risk, such as hereditary genetic disorders, obesity during adolescence, being tall and exposure to high doses of ionizing radiation [2-4, 6, 19]. Certain genome-wide association studies (GWAS) have reported that single nucleotide polymorphisms are associated with glioma susceptibility [6, 19]. However, the additional factors that contribute to glioma susceptibility require further investigation.

Disturbances in the control of cell cycle play an important role in cancer formation [20]. Cyclin D1 is a key regulatory protein in the cell cycle, playing a critical role in the transition from G1 to S phase of the cell cycle. Cyclin D1 regulates cell cycle progression by activating cyclin dependent kinase 4 (CDK4) and cyclin dependent kinase 6 (CDK6), which in turn phosphorylate the retinoblastoma (Rb) protein. The phosphorylation of Rb releases the transcriptional factor E2F, which then activates a number of downstream genes necessary for cell cycle progression. This event lead to progression through G1/S transition [21-23]. Cyclin D1 is encoded by the CCND1 gene located on chromosome 11q13. Betticher et al. identified G870A polymorphism in exon 4 of the CCND1 gene [24]. This polymorphism doesn't cause to an amino acid change, but CCND1 mRNA is alternatively spliced to produce two transcripts. The CCND1 G870 allele splices transcript a, whereas the CCND1 870A allele mainly splices transcript b. The protein encoded by transcript b, cyclin D1b, however, lacks the degradation

signal encoded by exon 5 and hence may have a longer half-life, resulting in deregulated cell proliferation. Increased expression of cyclin D1 may lead to premature cell passage through G1/S transition, which result in the propagation of unrepaired DNA damage, accumulation of genetic errors, and a selective growth advantage for the altered cells [17, 25-27].

A number of studies have suggested that the CCND1 G870A polymorphism is associated with susceptibility to various cancers, including esophageal adenocarcinoma, breast cancer, lung cancer, endometrial cancer, prostate cancer, bladder cancer, hepatocellular carcinoma, colorectal cancer, and cervical cancer [8-14]. Previously, a few studies have investigated the association between CCND1 G870A polymorphism and the risk of glioma, however, the conclusions were not consistent [15-17]. In the present study, we found that the A allele frequency was higher in cases than that in controls, and this difference was statistically significant. The frequencies of the GG, AG, and AA genotypes were 36.53%, 28.14%, and 35.33% in the glioma cases, respectively, and 45.56%, 36.11%, and 18.33% in the controls, respectively, and the difference was statistically significant. To evaluate the risk of glioma according to the CCND1 G870A allele and genotype, logistic regression analysis was conducted with adjustment for age, sex, smoking status and alcohol use. Using the G allele as the reference allele, the subjects carrying the A allele had 3.926-fold increase in the risk of glioma. Compared to individuals with the GG genotype, individuals with the AA genotype exhibited significantly increased glioma risk.

In conclusion, our results suggest that the CCND1 G870A polymorphism may contribute to the susceptibility to glioma in Chinese population. And larger prospective studies are needed to elucidate the precise role of the CCND1 G870A polymorphism in glioma.

Disclosure of conflict of interest

None.

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