Association of genetic variants in the CART gene with glioma susceptibility in a Chinese population

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ABSTRACT

Glioma, which is a rare and highly fatal brain cancer, has been studied for many decades. However, only a few etiological factors have been established. Genetic factors play an essential roles in the development of gliomas and are key component of preventive oncology. However, only a small proportion of the genetic effect has been yet established. In current study, we systematically evaluated whether genetic variants of CART gene, which generates multiple biologically active peptides, contribute to susceptibility of gliomas among Chinese people with a two-stage, case–control study. In stage I, we found rs2239670 (Allele A *vs* G: OR = 1.33; 95% CI = 1.03-1.70; *P* = 0.026) and rs11575893 (Allele T *vs* C: OR = 1.29; 95% CI = 1.01-1.65; *P* = 0.040) were significantly associated with increased glioma susceptibility. Then the two SNPs were significantly replicated in an independent stage. When pooled together, both rs2239670 (Allele A *vs* G: OR = 1.27; 95% CI = 1.10-1.46; *P* = 0.001) and rs11575893 (Allele T *vs* C: OR = 1.25; 95% CI = 1.09-1.45; *P* = 0.002) were significant associated with increased glioma susceptibility. Our data suggest that the genetic variants in the CART gene potentially predispose their carriers to gliomas.

INTRODUCTION

Gliomas make up approximately 80% of all malignant brain tumors [1]. Although relatively uncommon in the general population, gliomas owns high morbidity and mortality [2-5]. In China, the estimated incidence and mortality numbers of brain tumors, which were mainly composed by gliomas, are 101.6 and 61.0 thousands in 2015, respectively [6], while the correspondent numbers in United States are 27,770 and 16,050 in 2016, respectively [7]. Gliomas were composes of several subtypes, mainly including Glioblastomas, which account for approximately 60 to 70%, anaplastic astrocytomas, which account for 10 to 15%, and anaplastic oligodendrogliomas and anaplastic oligoastrocytomas, which account for 10% [3, 8-11]. To present, only a small proportion of the genetic factors for gliomas has been yet established, including results of familial studies, candidate gene studies, and genome-wide association studies (GWASs) [12-16]. Understanding of the genetic basis is a key component of preventive oncology.

Cocaine and amphetamine regulated transcript (CART) gene encodes cocaine and amphetamine regulated transcript prepropeptide (CARTPT), which generates multiple biologically active peptides [17-19]. These peptides play an important role in cancers, schizophrenia, alcohol use disorders, nicotine dependence, methamphetamine dependence, and obesity [20-25]. Genetic association studies could provide solid evidence for the potential role of CART gene in human malignant gliomas. However, no studies have evaluated that whether genetic variants of CART contribute to risk of gliomas. In current, we performed a two-stage, case-control study to investigated the association between tag SNPs in the CART gene and gliomas susceptibility among Chinese population.

Table 1: Comparison of gliomas patients and controls by selective characteristics

	Stage I			Stage II		
Variables	**Cases (n=400)**	**Controls (n=400)**	**P value**	**Cases (n=800)**	**Controls (n=800)**	***P* value**
Age (years)	48.2±3.8	48.5±3.3	0.233	45.3±4.2	45.1±4.7	0.370
Gender (male)	244 (61.0%)	248 (62.0%)	0.771	480 (60.0%)	468 (58.5%)	0.542
Family history of cancer	86 (21.5%)	75 (18.8%)	0.332	160 (20.0%)	131 (16.4%)	0.060
Smoking status						
Ever	100 (25.0%)	97 (24.2%)	0.806	162 (20.2%)	132 (16.5%)	0.053
Never	300 (75.0%)	303 (75.8%)		638 (79.8%)	668 (83.5%)	
Alcohol status						
Ever	114 (28.5%)	104 (26.0%)	0.435	238 (29.8%)	160 (20.0%)	***P*<0.001**
Never	326 (81.5%)	336 (84.0%)		562 (70.2%)	640 (80.0%)	

Table 2: Association between CART gene polymorphisms and the risk of gliomas in stage I

		Genotype (N)			OR (95 % CI) [1]			
SNPs	Subject	11	12	22	2 vs 1	12 vs 11	22 vs 11	***P* value**
rs2239670	Case	257	115	28	1.33 (1.03-1.70)	1.20 (0.88-1.65)	1.91 (1.02-3.57)	**0.026**
	Control	280	104	16				
rs3846659	Case	250	128	22	1.12 (0.88-1.43)	1.10 (0.81-1.50)	1.28 (0.67-2.43)	0.353
	Control	261	121	18				
rs11575893	Case	246	130	24	1.29 (1.01-1.65)	1.24 (0.92-1.68)	1.75 (0.91-3.40)	**0.040**
	Control	270	115	15				
rs6894772	Case	285	105	10	1.05 (0.80-1.38)	1.12 (0.81-1.54)	0.85 (0.36-2.00)	0.729
	Control	292	96	12				

[1] adjusted for Age, gender, family history of cancer, smoking status and alcohol status

RESULTS

Demographic characteristics of the subjects

In stage I, a total of 400 gliomas patients and 400 healthy control were recruited, while 800 gliomas patients and 800 healthy control were recruited in stage II. Table 1 shows the comparison of gliomas patients and controls by selective characteristics in both stage I and stage II. No significant difference in the distribution of age, gender, family history of cancer, and smoking status was found between the gliomas patients and healthy controls in two stages ($P > 0.05$). Only the gliomas patients are more likely to be drinkers in stage II ($P < 0.001$).

Associations between CART gene polymorphisms and glioma susceptibility in the discovery stage

According to the selection criteria, four tagSNPs (rs2239670, rs3846659, rs11575893, and rs6894772) are selected using SNPinfo. Table 2 presents the genotype frequencies of the selected SNPs and their associations with glioma susceptibility. None of the genotype distributions for the four tag SNPs departed from the HWE ($P > 0.05$). Our results showed that rs2239670 (Allele A *vs* G: OR = 1.33; 95% CI = 1.03-1.70; $P = 0.026$) and rs11575893 (Allele T *vs* C: OR = 1.29; 95% CI = 1.01-1.65; $P = 0.040$) were significantly associated with increased glioma susceptibility. Compared with the carriers of genotype GG of rs2239670, those of genotype AA (OR = 1.91; 95% CI = 1.02-3.57) had significantly increased glioma susceptibility. However, no significant associations were found for rs3846659 and rs6894772.

Validation analysis of the association between selected CART SNPs and glioma susceptibility

To validate the results above, we evaluated the associations of CART rs2239670 and rs11575893 with glioma susceptibility was evaluated in stage II (Table 3). The genotype distribution of rs2239670 and rs11575893 in controls were also consistent with the HWE ($P > 0.05$). The positive trend for rs2239670 ($OR_{trend} = 1.24$; 95% CI = 1.04-1.47; $P = 0.015$) and rs11575893 ($OR_{trend} = 1.23$; 95% CI = 1.04-1.47; $P = 0.018$) was significantly replicated in Stage II. When pooled the results of the two stages together, both rs2239670 (Allele A *vs* G: OR = 1.27; 95% CI = 1.10-1.46; $P = 0.001$) and rs11575893 (Allele T *vs* C: OR = 1.25; 95% CI = 1.09-1.45; $P = 0.002$) were significant associated with increased glioma susceptibility. For rs2239670, compared with the carriers of genotype

Table 3: Genotype frequencies of CART rs2239670, rs11575893 and association with risk of gliomas in stage II and the merged results

	Cases (n=800)	Controls (n=800)	OR[1] (95% CIs)	*P* value
rs2239670				
Stage II				
GG	499	536	Reference	
AG	251	232	1.16 (0.94-1.44)	
AA	50	32	1.68 (1.06-2.65)	
Additive model			1.24 (1.04-1.47)	**0.015**
Merged results				
GG	756	816	Reference	
AG	366	336	1.17 (0.98-1.40)	
AA	78	48	1.75 (1.21-2.53)	
Additive model			1.27 (1.10-1.46)	**0.001**
rs11575893				
Stage II				
CC	506	545	Reference	
CT	250	225	1.20 (0.96-1.49)	
TT	44	30	1.58 (0.98-2.54)	
Additive model			1.23 (1.04-1.47)	**0.018**
Merged results				
CC	752	815	Reference	
CT	380	340	1.21 (1.01-1.44)	
TT	68	45	1.64 (1.11-2.41)	
Additive model			1.25 (1.09-1.45)	**0.002**

[1] adjusted for Age, gender, family history of cancer, smoking status and alcohol status

Table 4: Stratified analyses of CART rs2239670, rs11575893 with risk of gliomas

	rs2239670		rs11575893	
	OR[1] (95% CIs)	*P* value	OR[1] (95% CIs)	*P* value
Smoking status				
Ever	1.27 (0.92-1.74)	0.143	1.25 (0.91-1.72)	0.161
Never	1.27 (1.08-1.48)	0.004	1.25 (1.07-1.47)	0.005
Alcohol status				
Ever	1.27 (0.95-1.68)	0.104	1.25 (0.94-1.67)	0.120
Never	1.27 (1.07-1.49)	0.005	1.25 (1.06-1.48)	0.007

[1] adjusted for Age, gender, and family history of cancer

GG, those of genotype AA (OR = 1.75; 95% CI = 1.21-2.53) had significantly increased glioma susceptibility. While for rs11575893, compared with the carriers of genotype CC, both those of genotype CT (OR = 1.21; 95% CI = 1.01-1.44) and TT (OR = 1.64; 95% CI = 1.11-2.41) had significantly increased glioma susceptibility. We also explored the effect modification of alcohol status and smoking status (Table 4), however, the results didn't change materially.

DISCUSSION

In this study, a two-stage, case-control study was applied to systematically evaluated whether genetic variants of CART gene contribute to susceptibility of gliomas among Chinese population. We identified that both rs2239670 and rs11575893 were significant associated with increased glioma susceptibility. To our knowledge, this should be the first study which aims to evaluated the association between genetic variants of CART gene and susceptibility of gliomas.

Except that exposure to ionizing radiation has been

established as a risk factor, most underlying cause for gliomas has not been identified [26-30]. Genetic factors, which have been explored for many decades, play an essential roles in the development of gliomas and are key component of preventive oncology, however, only a small proportion of the genetic effect has been yet established [1, 14, 31-34]. CART gene is located at 5q13.2 in humans, and is expressed in diverse brain structures, as well as endocrine tissues [35]. In 1999, Echwald et al first evaluated the potential effect of sequence variants CART gene in subjects with early onset obesity [25]. It was also found that hypothalamic CART expression could be involved a variety of neuroendocrine functions including food-intake [36]. Furthermore, CART gene was identified to be related with type 2 diabetes mellitus, hormone release, breast cancer, small bowel carcinoid tumors, neuroendocrine tumor, and so on [37-43].

In this study, we confirmed rs2239670 (Allele A *vs* G: OR = 1.27; 95% CI = 1.10-1.46; P = 0.001) and rs11575893 (Allele T *vs* C: OR = 1.25; 95% CI = 1.09-1.45; P = 0.002) were significant associated with increased glioma susceptibility. SNP rs11575893 is located at the 3' UTR region of the CART gene, while rs2239670 is an intronic variant. Using RegulomeDB [44, 45], both scores of rs2239670 and rs11575893 was identified to be 4, which means the SNPs could affect TF binding and DNase peak. When analyzed by HaploReg v4.1 [46], results showed that rs2239670 could alter regulatory motifs, including TATA,YY1,ZBTB7A, and could bind proteins including NRSF, HDAC2, RAD21 SIN3AK20, and ZNF263. While for rs11575893, motifs GCNF,Hand1,Myc could be changed, and it also could bind NRSF, ZNF263, and SIN3AK20. NRSF and ZNF263 are important modulator of malignant progression [47, 48]. Overall, our findings provided evidence for the important role of SNPs in CART gene in the tumorigenesis of gliomas.

Our study has several strengths. First, the two-stage study design provides a solid and trustable conclusion; second, the large sample size provides enough statistical power to detect such moderate associations. When interpreting the results of this study, several limitations should also be considered. First, selection and information bias might be unavoidable due to the natural of case-control study design, although the controls have been matched by age, gender, and race. Second, we still have not enough sample size to detect gene-environment interactions in current study. Third, it is uncertain whether our findings can be generalized to other populations due to the samples recruited in this study was restricted to Chinese population.

In conclusion, this study found that both CART rs2239670 and rs11575893 were significant associated with increased glioma risk using a two-stage, case-control study with a large sample size. The results suggest that the rs2239670 and rs11575893 may be used as biomarkers for prediction and screening of lung cancer. Validations with larger population-based studies in different ethnic groups and mechanism studies are warranted to further interpret the findings.

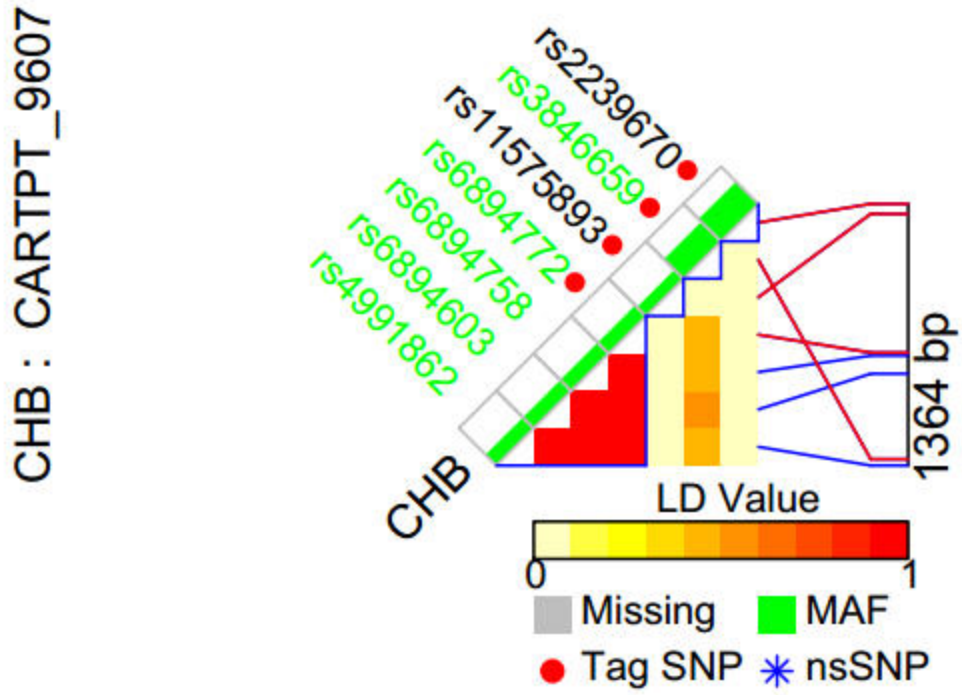

Figure 1:

MATERIALS AND METHODS

Subjects

Totally included in this study were 1,200 genetically unrelated gliomas cases from Tangdu Hospital, which were newly diagnosed and histopathologically confirmed patients until November 2015, as well as a total of 1,200 age-, gender-, race-matched, cancer-free healthy controls which were recruited from the same hospital at the same period. All the participants have no previous history of cancer and CNS-related diseases. Face to face interviews were performed using unified questionnaires to collect data on demographic characteristics and potential glioma susceptibility factors. After the interview, 5 ml peripheral blood sample were collected for each participant. The study protocol was approved by the Institutional Review Board of Tangdu Hospital and all of the participants provided written informed consent by themselves or their guardians.

SNP selection and genotyping

The tag SNPs of CART gene and its 1kb flanking region were selected using SNPinfo (http://snpinfo.niehs.nih.gov/) based on the criteria of minor allele frequency(MAF) >5% for Chinese Han subjects; Four tag SNPs in the CART gene that met the criteria were chosen in this study (Figure 1). Genomic DNAs were extracted by Qiagen DNA blood kit following the manufacturer's protocols. The SNP genotyping was performed by Sequenom MassArray iPLEX platform (Sequenom Inc., San Diego, CA, USA). Approximately 10% of the samples were randomly selected and genotyped with sequencing to validate the accuracy of genotyping results. A concordance of 100% for the quality control samples confirmed the liability of the Sequenom MassArray iPLEX platform.

Statistical analyses

The difference in gender, family history of cancer, smoking status and alcohol status between gliomas patients and healthy controls were evaluated using the chi-square test, while the difference in age between gliomas patients and healthy controls were tested by student's paired t test. Goodness-of-fit $\chi 2$ test were used to evaluate the potential departure from Hardy-Weinberg equilibrium (HWE) of genotypic frequencies in controls for each SNP. Odds ratios and corresponding 95% confidence intervals (CIs) were used to estimate the association between selected polymorphisms and glioma susceptibility. Adjusted ORs by age, gender, family history of cancer, smoking status and alcohol status were calculated using multivariate analysis with unconditional logistic regression. All the data was analyzed with SPSS software version 13.0 (SPSS Inc, Chicago, IL, USA). All statistical tests were two-sided, with a significance level of $P < 0.05$.

CONFLICTS OF INTEREST

The authors declare no competing financial interests.

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