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Original Article

Association of HLA-DMA rs1063478 polymorphism and gene-environment interactions with glioma

Chuanjun Guo[1,2], Xin Li[2], Yilei Xiao[2], Xueyuan Li[2], Zipeng Zhu[2], Fengyang Geng[2], Zhongmin Li[2], Shugan Zhu[1]

[1]Department of Neurosurgery, Qilu Hospital, Shandong University, Jinan 250012, Shandong, China; [2]Department of Neurosurgery, Liaocheng People's Hospital, Liaocheng 252000, Shandong, China

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Abstract: Objective: Glioma is one of the most common brain malignant tumors. Its occurrence results from the interactions between environmental and genetic factors. The purpose of the study was to investigate the relationship of human leukocyte antigen DMA (*HLA-DMA)* gene polymorphism and gene-environment interactions with the glioma susceptibility. Methods: *HLA-DMA* rs1063478 polymorphism was tested by the method of polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) in 90 glioma patients and 110 healthy controls. Chi-square test and binary regression analysis were adopted to detect the association between *HLA-DMA* rs1063478 polymorphism and glioma. Gene-environment interactions were explored by case-only approach. Odd ratios (ORs) and 95% confidence intervals (95% CIs) were used to represent the glioma susceptibility. Results: Rs1063478 TT genotype and CT+TT genotypes could increase glioma susceptibility, respectively. After being regulated by environmental factors, only CT+TT genotypes increased the susceptibility of glioma (OR=1.984, 95% CI=1.084-3.630). Gene-environment interaction analysis showed that there was interaction between rs1063478 with ionizing radiation (OR=5.359, 95% CI=1.13-25.797). Conclusions: *HLA-DMA* rs1063478 polymorphism is related to glioma susceptibility. Besides, it can make the susceptibility of glioma rise along with ionizing radiation.

Keywords: Glioma, *HLA-DMA,* polymorphism, gene-environment interactions

Introduction

Glioma is one of the most common primary brain malignant tumors in adults. Up to now, the pathogenesis of glioma has not been fully figured out. Among the causes, the exposure to chemical toxins and ionizing radiation is generally accepted, but they can merely explain a small portion of glioma pathogenesis. Only a small part of populations exposed to ionizing radiation and chemical toxins will finally suffer from glioma. All of this indicated the important role of individual susceptibility in the development of glioma. Besides, researches have shown that the occurrence of glioma is related to relevant genes of tumors [1-5]. Same with other tumors, it is possible that the gene-environment interactions are involved in the incidence of glioma [6].

Although the glioma pathogenesis was still unknown, many researchers suggested that glioma was correlated with autoimmunity [7-10]. Besides, other researchers have confirmed that human leukocyte antigen-DM alpha (*HLA-DMA*) gene participates in immune response of cancers and multiple diseases [11-16]. However, there are few researches about *HLA-DMA* with glioma. So we initiated our study using *HLA-DMA* gene. HLA-DM protein belongs to major histocompatibility complex (MHC) class II. It locate in intracellular vesicles, act as a molecular chaperones of antigen transfer process [17], and has a closely association with human immune system. *HLA-DMA* gene was located at sixth choromosome p21.3, and had five exons. It encodes the alpha chain of HLA-DM.

As far as we know, environmental factors affect the occurrence and development of glioma [18-21], and gene-environment interactions also correlate with glioma [6, 22]. But there is no research focused on *HLA-DMA* gene and environment interactions on glioma. In order to detect the correlation between the interactions and glioma susceptibility among Chinese Han

Table 1. Basic information of subjects

Variation		Cases n=90 (%)	Controls n=110 (%)	χ^2	*P*
Age (year)	≤50	18 (20.0)	23 (20.9)	0.060	0.970
	50-60	37 (41.1)	46 (41.8)		
	≥60	35 (38.9)	41 (37.3)		
Gender	Male	55 (61.1)	69 (62.7)	0.055	0.815
	Female	35 (38.9)	41 (37.3)		
Smoking	Yes	31 (34.4)	42 (38.2)	0.298	0.585
	No	59 (65.6)	68 (61.8)		
Drinking	Yes	38 (42.2)	50 (45.5)	0.210	0.674
	No	52 (57.8)	60 (54.5)		
Glioma family history	Yes	10 (11.1)	1 (0.9)	9.912	0.002
	No	80 (88.9)	109 (99.1)		
Chemical toxin	Yes	9 (10.0)	1 (0.9)	8.612	0.003
	No	81 (90.0)	109 (99.1)		
Ionizing radiation	Yes	13 (14.4)	2 (1.8)	11.375	0.001
	No	77 (85.6)	108 (98.2)		

Notes: Smoking: more than 1 cigarette per day last for 1 year, or cigarette consumed is ≥18 packs per year. Drinking: drinking frequency is ≥2 times per week, the consumption of liquor per time is ≥50 g or of other alcohols (beer, yellow rice wine or fruit wine) ≥500 ml.

Table 2. Relationship of rs1063478 polymorphism with glioma

Variation	Cases	Controls	OR (95% CI)*	OR (95% CI)**
rs1063478				
CC	40	68	-	reference
CT	38	36	1.794 (0.985-3.271)	
TT	12	6	3.400 (1.184-9.764)	
CT+TT	50	42	2.024 (1.149-3.566)	1.984 (1.084-3.630)

Notes: *represents the unadjusted OR values before correcting the confounding factors such as age, gender, smoking history, family history of glioma, chemical toxins and ionizing radiation, while **manifests the adjusted OR values after correcting these factors.

population, we explore the interaction of *HLA-DMA* rs1063478 polymorphism and environmental factors on glioma, with method of case-only study.

Materials and methods

Medical records

90 inpatients diagnosed as glioma in Affiliated Hospital of Liaocheng People's Hospital were enrolled as cases. Among them there were 55 males and 35 females with a median age of 56.38±6.32 years, aging 16~64 years. 110 healthy individuals who check up healthy examination in the same hospital at the same period were recruited as controls. Among them there were 69 males and 41 females with a median age of (58.05±7.13) years, aging 19~68 years. All participants had no blood relationship. The subjects were interviewed face to face with standardized structured questionnaire containing basic demographic data, smoking and drinking status, family history of glioma, the exposure to chemical toxins and ionizing radiation and etc. All persons in the trial gave informed consents. This study was carried out with the permission of the Ethis Committee of Affiliated Hospital of Liaocheng People's Hospital.

DNA extraction

5 ml peripheral venous blood was collected from every fasting subject, and then was conducted with anticoagulation by using ethylene diamine tetraacetic acid-Na2 (EDTA-Na2). The method of phenol-chloroform was used to extract genome DNA.

Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis

The designed primers were synthesized by Affiliated Hospital of Liaocheng People's Hospital biological engineering Co., Ltd. Primer sequences were as follows: forward: 5'-CCA CCA GGG TTT CCT ATC GC-3'; reverse: 5'-GCC AAA CTC CAG GGG CTT CA-3'. 25 μl reaction amplification mixture included 2.5 μl of 10 × PCR buffer, 0.5 μl of dNTPs (2.5 mol/L), each 0.5 μl of forward and reverse primers (10 μmol/L), 0.3 U of Taq DNA polymerase, 1 μl (100 ng/μl) of genome DNA template and 19.7 μl of double distilled water. The mixture was put in FQD-48A PCR instrument for amplification. The reaction initially performed with pre-degeneration at 95°C for 3 min, followed by 40 circles of degeneration at 94°C for 40 s, annealing at

Table 3. The analysis of gene-environment interactions

Environment factor	CC		CT+TT		*P*	OR (95% CI)
	Yes	No	Yes	No		
Chemical toxin	2	38	7	43	0.289	3.093 (0.065-15.801)
Ionizing radiation	2	38	11	39	0.033	5.359 (1.13-25.797)

55°C for 40 s and extension at 72°C for 1 min, finally extension at 72°C for 7 min.

PCR products with the fragment length of 279 bp were digested by corresponding restriction enzyme *AvrII*. Then 10 μl enzyme digestion system was incubated at 37°C for 15 h. The result of enzyme digestion was tested by 2% agarose gel electrophoresis with 150 V for 20 min.

Definition of environmental factors

All patients with glioma were conducted with the survey of environmental factors, mainly including chemical toxins and ionizing radiation. Firstly, contacted or engaged in jobs relevant to chemical toxins. Secondly, radiation therapy had been accepted or ionizing radiation contacted due to occupational exposure. All these environmental risk factors had occurred before the diagnosis of glioma.

Statistical treatment

PLINK1.07 software was used to detect Hardy Weinberg equilibrium (HWE) in case and control groups. Chi-square test was used for analyzing the features of the participants, as well as detecting the interactions of gene-environment. Environment factors were controlled by Logistic regression analysis to obtain adjusted odd ratios (ORs) and 95% confidence intervals (95% CI). Statistic significant existed when $P<0.05$.

Results

Features of subjects

Basic information of the 90 glioma patients and 110 healthy controls was shown in **Table 1**. From the table we could see the age of most subjects was more than 50 years, and the number of male was also more than female. Factors, such as age, gender, histories of smoking and drinking had no significant differences among the two groups ($P>0.05$). 10 patients with glioma family history accounted for 11.1% in cases, which had apparent higher proportion than controls (0.9%), and the difference had statistical significance ($P=0.002$). Compared with the controls (0.9%), there were 9 cases (10.0%) with contact history of chemical toxins, that was possessed with statistical significance ($P=0.003$). Owing to radiation therapy in hospital (4) and professional exposure (9), there were 13 patients with ionizing radiation in case group. The proportion of people suffered from ionizing radiation showed significant difference in the two groups (cases 14.4% vs. controls 1.8%; $P=0.001$).

HLA-DMA rs1063478 polymorphism related to glioma risk

Genotype and allele distributions in case and control groups were according with HWE. TT mutant homozygous genotype frequency in case group was higher than that in control group, with 3.40 times in terms of increasing the risk of glioma. Heterozygous genotype CT had no significant association with glioma risk. CT+TT genotypes had more glioma risk than CC genotype (OR=2.204, 95% CI=1.149-3.566). After adjusted with environment factors, CT+TT genotypes still increased the risk of glioma (OR=1.984, 95% CI=1.084-3.630) (**Table 2**).

Gene-environment interactions

In the basis of the case only analysis, the results of gene-environment interactions were obtained and shown in **Table 3**. The results demonstrated that *HLA-DMA* rs1063478 polymorphism had interaction with ionizing radiation, and the interaction enhanced the risk of glioma ($P=0.033$, OR=5.359, 95% CI=1.13-25.797). But chemical toxin had no significant association with glioma.

Discussion

Glioma is the commonest primary malignant tumor in central nervous system. It is accounts for 50%-60% of intracranial tumors, 2% of all the malignant tumors for adults [23, 24], presenting with infiltrative growth, poor prognosis. The pathological features of glioma were significantly different from other malignant tumors. Due to good invasion ability, glioma had high tends of recurrence. Even though the patients are conducted with comprehensive treatment of radiotherapy and chemotherapy, the survival

time of patients with lower-level glioma is 3-5 years while the high-level is 1-2 years. It has been confirmed that glioma is caused by complex factors of inheritance and environment. However, knowledge about its pathogenesis and susceptible genes is limited. Therefore, it is imperative to look for the susceptible genes for it, which will be meaningful for the prevention and diagnosis of glioma.

HLA-DMA is closely connected with human immune system. Many cancers are caused by the drawback of human immune surveillance. Owing to these reasons, *HLA-DMA* gene is connected with the incidence of many cancers [25, 26]. A lot of literatures explore the structure and function of *HLA-DMA* gene. Traditionally, *HLA* gene is divided into 3 domains in order, namely *HLA*-I, *HLA*-II and *HLA*-III gene regions [27]. *HLA-DM* gene is located between *HLA-DP* and *HLA-DQ* at the class II gene region of *HLA*. It is first discovered by Cho et al. in 1991 [28], and it is found that *HLA-DM* is a atypical gene. *HLA-DMA* gene contained five exons, and the 1-4 exons were involved in transcription. Polymorphisms of classical human *HLA*-II gene are located in the second exons, but the second exon of *HLA-DM* gene is highly conserved. The polymorphisms of *HLA-DM* gene are located in the third exons, such as rs1063478. Various researches focused on the association between *HLA-DM* polymorphisms and many diseases [29, 30]. There also exist many researches focused on rs1063478 polymorphism [31-33]. Rs1063478 is a missense mutation, and the amino acids alter from Val to Ile. This mutation alters the function of HLA-DMA in antigen processing and presenting [31].

In present study we explored the association between rs1063478 polymorphism with glioma risk. Besides, we also investigated whether the interactions of rs1063478 polymorphism and environmental factors affected glioma susceptibility. That should be contributed to investigate the pathogenesis of glioma. In our study we found that glioma family history, chemical toxin and ionizing radiation were significantly associated with glioma risk. That was conformed with the research conducted by Morgan et al., suggesting the mobile phone radiation might increase the glioma risk [18]. No significant association existed in glioma risk and other features, such as age, gender, smoking and drinking. According to the genotype analysis, we suggested that CT+TT genotypes had increased the risk of glioma risk about 1.984 times. The result was accorded with the study on psoriasis and Behcet's disease [32]. But it was different from previous research, which indicated rs1063478 T allele offered protective effect on hepatitis C virus (HCV) infection [33]. Then we analyzed the gene-environment interactions. The results showed that the interactions between rs1063478 polymorphism and ionizing radiation could make glioma risk rise, with the OR value was 5.359. But the interactions between rs1063478 polymorphism and chemical toxin had no statistical significance. The results suggested that the gene-environment interactions obviously increased the glioma risk.

Environment risk factors can be avoided; current study suggested that keep away from ionizing radiation could decrease the glioma risk. Although we got the meaningful results, the glioma pathogenesis still unknown. In order to explore the pathogenesis, a well designed research will be need.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Shugan Zhu, Department of Neurosurgery, Qilu Hospital, Shandong University, No.107, Wenhua West Road, Jinan 250012, Shandong, China. E-mail: gcj0123456@163.com

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