RESEARCH ARTICLE

Xeroderma Pigmentosum Complementation Group F Polymorphisms Influence Risk of Glioma

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Abstract

We conducted an exploratory investigation of whether variation in six common SNPs of xeroderma pigmentosum complementation group F (XPF) is associated with risk of glioma in a Chinese population. Six single nucleotide polymorphisms (SNPs) were genotyped in 207 glioma cases and 236 cancer-free controls by a 384-well plate format on the Sequenom MassARRAY platform (Sequenom, San Diego, USA). The rs1800067 G and rs2276466 G allele frequencies were significantly higher in the glioma group than controls. Individuals with the rs1800067 GG genotype were at greater risk of glioma when compared with the A/A genotype in the codominant model, with an OR (95% CI) of 2.63 (1.04-7.25). The rs2276466 polymorphism was significantly associated with moderate increased risk of glioma in codominant and dominant models, with ORs (95% CI) of 1.90 (1.05-3.44) and 1.55 (1.07-2.47), respectively. The combination genotype of rs1800067 G and rs2276466 G alleles was associated with a reduced risk of glioma (OR=0.44, 95% CI=0.19-0.98). These findings indicate that genetic variants of the XPF gene have critical functions in the development of glioma.

Keywords: Xeroderma pigmentosum complementation group F - single nucleotide polymorphisms - glioma

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Introduction

Malignant gliomas account for approximately 80% of all the primary malignant brain tumors. Previous many studies have been conducted on the etiology of glioma, but they has yielded few consistent finding. The only confirmed environment risk factor of glioma is exposure to high doses of ionizing radiation (Schwartzbaum et al., 2006; Bondy et al., 2008; Ostrom and Barnholtz-Sloan, 2011). Previous studies on glioma focuses on identifying these inherited factors which can modify the susceptibility of cancer (Bethke et al., 2008; Shete et al., 2009; Wrensch et al., 2009).

Nucleotide excision repair (NER) is the most versatile DNA repair mechanism pathway responsible for removing a wide variety of DNA lesions, such as bukly adducts, cross links, oxidative DNA damage, alkylating damage and thymidine dimmers (Wood et al., 2001).

One of the NER genes, xeroderma pigmentosum complementation group F (XPF), is located on chromosome 16p13.12 and contains 11 exons and spans approximately 28.2 kb, and is a key component involved in the 5' incision made during NER (Liu et al., 1993; Wood et al., 2001). The XPF protein consists of 916 amino acids, containing an XPF domain that is one of the nuclease family, in whichessential meiotic endonuclease 1(EME1) acts as an essential component of a Holiday junction resolvase to interact with MUS81 (Tripsianes et al., 2005; Tsodikov et al., 2005). Moreover, the XPF domain is a critical for forming a tight complex with ERCC1 which responsible for the 5'-primer incision during the DNA excision repair mechanism (Tripsianes et al., 2005; Tsodikov et al., 2005). Up to now, a total of 580 single nucleotide polymorphisms (SNPs) in the XPF gene have been reported according to the dbSNP database (http://snpinfo.niehs.nih.gov/), some of which have been reported to be associated with risk of several kinds of cancers, such as breast cancer, endometrial cancer, colorectal cancer, head and neck cancer, lung cancer and skin cancers (Huang et al., 2006; Han et al., 2009; Doherty et al., 2011). However, only one study explores the role of two SNPs of ERCC4 in the risk of glioma in USA, so we reported the predictive role of four XPF SNPs in the risk of glioma.

Therefore, this study conducts an exploratory investigation of whether variation in six common SNPs of XPF is association with risk of glioma. Our study indicated important evidence for the association between polymorphisms in six XPF SNPs and risk of glioma, and

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the role of gene-gene interaction in the cancer risk.

Materials and Methods

Study population

In our study population, a total of 207 patients with glioma between November 2008 and December 2011 were recruited from an ongoing molecular epidemiological study at the Fourth Affiliated Hospital of Harbin Medical University in China. All glioma cases were diagnosed within one month and histologically confirmed, and none of them have a previous history of other cancer, prior chemotherapy or radiotherapy. There were no restrictions on selecting cases by age, sex or disease stage. The research protocol was approved by the ethics committees of the Fourth Affiliated Hospital of Harbin Medical University, and informed consent was obtained from all recruited subjects.

A random sample of 269 health individuals were selected between January 2009 to Decmber 2011 from Health Examination Center at the Fourth Affiliated Hospital of Harbin Medical University, and matched with cases by sex and age (±5 years). Subjects with chronic diseases, heart, lung liver kidney, brain, severe endocrinological, metabolic and nutritional diseases were excluded from our study. Finally, a total of 236 patients met the requirement and agreed to participate into our study, with participation rate of 87.7%.

Demographic data were collected through an face to face interview using a standardized epidemiological questionnaire, including age, sex, smoking and drinking status, family history of cancer. For patients, detailed clinical information was collected through a medical records or consultation from physicians.

SNPs selection and genotyping

Candidate SNPs in the XPF gene were selected from NCBI dbSNP database and SNPinfo with a MAF≥5% in the HapMap Asian population. The SNPs could affect transcription factor binding site (TFBS) activity in the putative promoter region, or influence the miRNA binding site activity. Finally, we chose six XPF SNPs, rs3136038, rs1799798, rs1800067, rs6498486, rs2276465 and rs2276466 according to the inclusion criteria. Genomic DNA was extracted from the whole blood using the method of phenol-chloroform extraction. Genotyping of the six SNPs was performed in a 384-well plate format on the Sequenom MassARRAY RS1000 platform with a standard protocol recommended by the manufacturer (Sequenom, San Diego, USA), and methods of polymerase chain reaction (PCR) and matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry technologies. PCR product was testified by on 1.0% agrose gel electrophoresis. A repeat analysis of a randomly chosen subgroup of 10% of the cases and controls was conducted for quality control, and the reproducibility was 100%.

Statistical analyses

Statistical analysis was performed using the SAS software (version 9.1.3; SAS Institute Inc., Cary, NC). All tests were two-sided, and P value less than 0.05 was considered as statistically significance. Continuous variables were shown as mean±SD and analyzed by students t test. Categorical variables were expressed as frequency and percentage and analyzed by chi-square test. Genotypic frequencies in control subjects for each SNP were tested for the Hardy-Weinberg equilibrium using chi-square test. Allele frequencies and genotype frequencies of each SNP between glioma cases and controls by means of a chi-square test and Student's t test. The genetic models, dominant and recessive models, were used and conducted using PLINK software to evaluate the associations between each SNP and glioma risk. For each polymorphism, odds ratios (ORs) and 95% confidence interval (CI) were calculated by unconditional logistic regression analysis adjusted for potential risk factors.

Results

Study subjects

A total of 207 glioma patients (140 Male, 67 female; median age at diagnosis, 51.2±11.4 years old) and 236 controls (154 Male, 82 female; median age at diagnosis, 50.7±11.2 years old) were included in the current study. Demographic characteristics of the cases and controls were shown in Table 1. Briefly, patients with glioma were significantly more likely to be male and had a history of ionizing radiation (IR) exposure and brain cancer in the first relatives than control subjects ($P < 0.05$ for all comparisons; Table 1).

Genotype distributions of the four SNPs are shown in

Table 1. Characteristics of the Study Population

Characteristics	Cases N=207	%	Controls N=236	%	χ^2	*P* value
Age (mean±SD)	(years) 51.2±11.4				50.7±11.2	
Sex						
Male	140	67.7	154	65.3	0.28	0.59
Female	67	32.3	82	34.7		
Smoking status						
Never	115	55.5	141	59.7		
Former	25	12.1	20	8.5	1.76	0.42
Current	67	32.4	75	31.8		
IR exposure history[1]						
Never	172	83.1	222	94.1		
Ever	35	16.9	14	5.9	8.1	<0.05
History of brain cancer in the first relatives						
No	196	94.7	235	99.6		
Yes	11	5.3	1	0.4	10.01	<0.05
Histological types[2]						
High grade glioma	111	53.6				
Medium grade glioma	47	22.7				
Low grade glioma	49	23.7				

[1]Including medical and occupational ionizing radiation exposure history. Medical ionizing radiation exposure defined as diagnostic (routine chest X-rays) and any radiotherapy for a medical problem of at least two years before diagnosis. Occupational ionizing radiation exposure included work as a pilot, flight attendant, astronaut, uranium miner, workers in the nuclear power industries, radiologists or X-ray medical worker, dentist or dental hygienist, and other participants who self-reported ionizing radiation exposure in the workplace. [2]High grade: glioblastoma; medium grade: anaplastic astrocytoma; low grade: oligodendroglioma, not-specified astrocytoma and mixed glioma

Table 2. In control subjects, the minor allele frequencies (MAFs) were consistent with published MAFs (available at http://www.ncbi.nlm.nih.gov/snp/), and in Hardy-Weinberg equilibrium. The rs1800067 and rs2276466 genotype frequencies were significantly different between the glioma cases and control groups, with the rs1800067 G allele and rs2276466 G allele frequencies significantly higher in the glioma group than controls (P<0.05). There were no significant between-group differences in the frequencies of rs3136038, rs1799798, rs6498486 and rs2276465 (Table 2).

Table 3 shows the results of multivariate logistic regression analysis of the effects of the six SNPs on glioma risk, adjusted for potential confounding factors (Table 3). Individuals with rs1800067 GG genotype were more likely to increase the risk of glioma when compared with A/A genotype in codominant model, with OR (95% CI) of 2.63 (1.04-7.25). Moreover, rs2276466 GG was significantly associated with moderate enhanced risk of glioma when compared with CC genotype in codominant model (OR=1.90, 95% CI=1.05-3.44), and variant of rs2276466 was associated with increased risk of glioma in dominant models, with ORs (95% CI) of 1.55 (1.07-2.47). A further association analysis was conducted to identify the interactions of two susceptibility-associated SNPs, rs1800067 and rs2276466, and their impact on glioma risk. The combination genotype of rs1800067 G allele and rs2276466 G allele was associated with a heavy risk of glioma (OR=0.44, 95% CI=0.19-0.98) (Table 4).

Table 2. Association Between Six XPF Polymorphisms and Glioma Risk

dbSNP	Major/Minor allele	MAF From dbSNP	MAF Case	MAF Control	Allele-specific OR(95% CI)	Allele-specific *P* value	P for HWE in controls
rs3136038	C/T	0.3324	35.2	34.3	1.05(0.79-1.40)	0.77	0.36
rs1799798	A/G	0.0975	12.7	10.9	1.19(0.81-1.87)	0.42	0.13
rs1800067	A/G	0.0311	17.9	12.7	1.49 (1.02-2.21)	0.03	0.86
rs6498486	A/C	0.2637	31.2	29.7	1.08(0.81-1.46)	0.63	0.09
rs2276465	A/G	0.2647	32.1	29.5	1.15(0.85-1.56)	0.39	0.1
rs2276466	C/G	0.2248	39.1	31.3	1.43(1.07-1.94)	0.01	0.45

Discussion

As far as we known, our study is the first attempt to examine the potential role of XPF SNPs on the risk of glioma. We found that rs1800067 and rs2276466 were strongly associated with glioma cancer risk, both individually and in combination. Although previous studies have been conducted on the association between DNA repaired genes and risk of glioma (Chen et al., 2012; Jacobs and Bracken, 2012; Wang et al., 2012), the role of variants of XPF in glioma risk has not been explored in Chinese population. These findings suggest that variants of rs1800067 and rs2276466 may be useful genetic susceptibility markers for glioma, allowing for identification of high-risk individuals and the development of targeted therapies.

Recently, identification of novel genetic variants for assessing the early risk of cancer is attracting great interest in researches on cancer risk worldwide (Liu et al., 2012; Pan et al., 2013; Walsh et al., 2013). Based on the genetic information, we could determine the genetic etiology of glioma, and the genetic factors can be used for identifying the high-risk individuals and perform targeting therapy according to individual's genetic make-up.

rs2276466 is located on the miRNA-binding site of the 3'UTR region. The functional significance of rs2276466 is still unclear, but increasing evidence indicated that

Table 4. Combination Effect of rs1800067 and rs2276466 on Glioma Risk

dbSNP	Cases N = 207	%	Controls N = 236	%	OR (95% CI)[1]	*P* value
rs1800067/ rs2276466						
AA/CC	61	29.5	86	36.4	-	-
G allele/CC	24	11.6	31	13.1	1.09 (0.55-2.2.14)	0.48
AA/G allele	88	42.5	99	41.9	1.25 (0.79-1.99)	0.82
G allele/G allele	34	16.4	20	8.5	2.39 (1.21-4.82)	0.03

[1]Adjusted for sex, age, IR exposure history and brain cancer history in the first relatives

Table 3. Genotype Frequencies and OR (95% CI) for Association Between XPF Polymorphisms and Glioma Risk

dbSNP	Major/Minor allele	Case	%	Control	%	Codominant model OR(95% CI)[1]	Codominant model *P* value	Dominant model OR(95% CI)	Dominant model *P* value
rs3136038	CC	90	43.4	105	44.5	-	-		
	CT	89	42.8	100	42.4	1.04(0.68-1.58)	0.85		
	TT	29	13.8	31	13.1	1.09(0.59-2.03)	0.76	1.04(0.70-1.54)	0.83
rs1799798	AA	164	79.1	190	80.5	-	-	-	
	AG	34	16.5	41	17.3	0.96(0.57-1.62)	0.88		
	GG	9	4.4	5	2.2	2.08(0.61-8.07)	0.18	1.08(0.66-1.77)	0.74
rs1800067	AA	149	72.2	182	77.2	-	-	-	
	AG	41	19.8	43	18.2	1.18(0.71-1.97)	0.49		
	GG	17	8	11	4.6	2.63(1.04-7.25)	0.02	1.54(0.92-2.81)	0.09
rs6498486	AA	105	50.5	124	52.5	-	-	-	
	AC	76	36.6	84	35.6	1.07(0.70-1.63)	0.75		
	CC	27	12.9	28	11.9	1.14(0.60-2.14)	0.67	1.08(0.73-1.61)	0.65
rs2276465	AA	102	49.1	124	52.5	-	-	-	
	AG	78	37.7	85	36.1	1.12(0.73-1.70)	0.59		
	GG	27	13.2	27	11.4	1.22(0.64-2.30)	0.51	1.16(0.78-1.69)	0.43
rs2276466	CC	85	41.2	115	48.7	-	-	-	
	CG	82	39.5	90	38.1	1.25(0.82-1.93)	0.27		
	GG	40	19.3	31	13.2	1.90(1.05-3.44)	0.02	1.55(1.07-2.47)	0.02

[1]Adjusted for sex, age, IR exposure history and brain cancer history in the first relatives

that SNPs which located at miRNA-binding sites or transcription factor binding site were more likely to influence the gene expression levels through modifying miRNA targeting activity or altering DNA binding properties, and subsequently increase the susceptibility to cancer (Knight, 2005; Blitzblau and Weidhaas, 2010; Pelletier and Weidhaas, 2010; Liu et al., 2011). In our study, we found variation of rs2276466 decreased the risk of glioma, which might be explained by the decreased expression of XPF mRNA and protein in lymphocytes to affect DNA repair capacity and modulating cancer susceptibility. The function of molecular mechanisms of the effects needs further investigation.

So far, no study has investigated the association between XPF SNPs and risk of glioma, while published studies focus on the association between variation of rs1800067 and various cancers, such as breast cancer, gastric cancer and head and neck cancer (Ding et al., 2011; He et al., 2012; Yu et al., 2012). However, the results are inconsistent. He et al. did not find significant association between two XPF SNPs and risk of gastric cancer in a large sample size study with 1125 gastric cancer cases and 1196 health controls (He et al., 2012). Another study which conducted in China with 1040 head and neck patients and 1040 health controls indicated that variants of rs2276466, rs3136038 and rs1800067 may increase the susceptibility to the cancer risk (Yu et al., 2012). Similarly, a meta-analysis found that variant of XPF rs1800067 may be a risk factor for developing breast cancer (Ding et al., 2011). In our study, we also found that GG genotype of rs1800067 increased the susceptibility to glioma, and the mutation of this gene might reduce the capacity of anticancer. It is hypothesis that GG genotype of rs1800067 might lower the expression of function of XPF, and thus individuals with G allele might have a lower capacity of double strand break repair capacity when compared with G/G genotype. Therefore, individuals with G allele genotype have not enough capacity to remove all double strand breaks on time and would have higher susceptibility to cancer.

Two limitations should be considered in our study. Firstly, our study selected controls from one hospital. This might be a selection bias since they controls were not a random sample of the general population and may not fully represent the underlying base population. Secondly, we only find light effect of the variants on the glioma risk, and no significant effect was found in other SNPs. This lack of significance could be either because the small sample size to limit the statistical power to detect a modest effect on cancer risk. Hence, further larger sample size studies are warranted to clarify the function of these SNPs.

Therefore, this case-control study indicated that rs2276466 and rs1800067 SNPs were associated with glioma risk in a Chinese population. These finding indicate that genetic variants of the XPF gene has a critical function on the development of glioma. Our study offers important insights into the molecular etiology of glioma.

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References

Bethke L, Webb E, Murray A, et al (2008). Comprehensive analysis of the role of DNA repair gene polymorphisms on risk of glioma. *Hum Mol Genet*, **17**, 800-5.

Blitzblau RC and Weidhaas JB (2010). MicroRNA binding-site polymorphisms as potential biomarkers of cancer risk. *Mol Diagn Ther*, **14**, 335-42.

Bondy ML, Scheurer ME, Malmer B, et al (2008). Brain tumor epidemiology: consensus from the Brain Tumor Epidemiology Consortium. *Cancer*, **113**, 1953-68.

Chen DQ, Yao DX, Zhao HY, Yang SJ (2012). DNA repair gene ERCC1 and XPD polymorphisms predict glioma susceptibility and prognosis. *Asian Pac J Cancer Prev*, **13**, 2791-4.

Ding DP, He XF, Zhang Y (2011). Lack of association between XPG Asp1104His and XPF Arg415Gln polymorphism and breast cancer risk: a meta-analysis of case-control studies. *Breast Cancer Res Treat*, **129**, 203-9.

Doherty JA, Weiss NS, Fish S, et al (2011). Polymorphisms in Nucleotide Excision Repair Genes and Endometrial Cancer Risk. *Cancer Epidemiol Biomarkers Prev*, **20**, 1873-82.

Han J, Haiman C, Niu T, et al (2009). Genetic variation in DNA repair pathway genes and premenopausal breast cancer risk. *Breast Cancer Res Treat*, **115**, 613-22.

He J, Xu Y, Qiu LX, et al (2012). Polymorphisms in ERCC1 and XPF genes and risk of gastric cancer in an eastern Chinese population. *PLoS One*, **7**, e49308.

Huang WY, Berndt SI, Kang D, et al. (2006). Nucleotide excision repair gene polymorphisms and risk of advanced colorectal adenoma: XPC polymorphisms modify smoking-related risk. *Cancer Epidemiol Biomarkers Prev*, **15**, 306-11.

Jacobs DI and Bracken MB (2012). Association between XRCC1 polymorphism 399 G- >A and glioma among Caucasians: a systematic review and meta-analysis. *BMC Med Genet*, **13**, 97.

Knight JC (2005). Regulatory polymorphisms underlying complex disease traits. *J Mol Med (Berl)*, **83**, 97-109.

Liu HB, Peng YP, Dou CW, et al (2012). Comprehensive study on associations between nine SNPs and glioma risk. A*sian Pac J Cancer Prev*, **13**, 4905-8.

Liu P, Siciliano J, White B, et al (1993). Regional mapping of human DNA excision repair gene ERCC4 to chromosome 16p13.13-p13.2. *Mutagenesis*, **8**, 199-205.

Liu Z, Wei S, Ma H, et al (2011). A functional variant at the miR-184 binding site in TNFAIP2 and risk of squamous cell carcinoma of the head and neck. *Carcinogenesis*, **32**, 1668-74.

Ostrom QT and Barnholtz-Sloan JS (2011). Current state of our knowledge on brain tumor epidemiology. *Curr Neurol Neurosci Rep*, **11**, 329-35.

Schwartzbaum JA, Fisher JL, Aldape KD, et al (2006). Epidemiology and molecular pathology of glioma. *Nat Clin Pract Neurol*, **2**, 494-503.

Tripsianes K, Folkers G, Ab E, et al (2005). The structure of the human ERCC1/XPF interaction domains reveals a complementary role for the two proteins in nucleotide excision repair. *Structure*, **13**, 1849-58.

Pan WR, Li G, Guan JH (2013). Polymorphisms in DNA repair genes and susceptibility to glioma in a chinese population. *Int J Mol Sci,* **14**, 3314-24.

Pelletier C and Weidhaas JB (2010). MicroRNA binding site polymorphisms as biomarkers of cancer risk. *Expert Rev Mol Diagn*, **10**, 817-29.

Shete S, Hosking FJ, Robertson LB, et al (2009). Genome-wide association study identifies five susceptibility loci for glioma. *Nat Genet*, **41**, 899-904.

Tsodikov OV, Enzlin JH, Scharer OD, Ellenberger T (2005). Crystal structure and DNA binding functions of ERCC1, a subunit of the DNA structure-specific endonuclease XPF-ERCC1. *Proc Natl Acad Sci U S A,* **102**, 11236-41.

Walsh KM, Anderson E, Hansen HM, et al (2013). Analysis of 60 reported glioma risk SNPs replicates published GWAS findings

but fails to replicate associations from published candidate-gene studies. *Genet Epidemiol*, **37**, 222-8.
Wang D, Hu Y, Gong H, Li J, Liu A (2012). Genetic polymorphisms in the DNA repair gene XRCC1 and susceptibility to glioma in a Han population in northeastern China: A case-control study. *Gene*, **509**, 223-7.
Wood RD, Mitchell M, Sgouros J, Lindahl T (2001). Human DNA repair genes. *Science*, **291**, 1284-9.
Wrensch M, Jenkins RB, Chang JS, et al (2009). Variants in the CDKN2B and RTEL1 regions are associated with high-grade glioma susceptibility. *Nat Genet*, **41**, 905-8.
Yu H, Liu Z, Huang YJ, et al (2012). Association between single nucleotide polymorphisms in ERCC4 and risk of squamous cell carcinoma of the head and neck. *PLoS One*, **7**, e41853.