Xeroderma Pigmentosum Complementation Group F Polymorphisms Influence Risk of Glioma

Hong-Bin Cheng, Chen Xie, Ru-You Zhang, Shao-Shan Hu, Zhi Wang, Wu Yue*

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

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 bulky 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'-primer 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 which essential 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
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 December 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% agarose 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.

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

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Cases N=207</th>
<th>%</th>
<th>Controls N=236</th>
<th>%</th>
<th>χ²</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mean±SD) (years) 51.2±11.4</td>
<td>50.7±11.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Male</td>
<td>140</td>
<td>67.7</td>
<td>154</td>
<td>65.3</td>
<td>0.28</td>
<td>0.59</td>
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<tr>
<td>Female</td>
<td>67</td>
<td>32.3</td>
<td>82</td>
<td>34.7</td>
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<tr>
<td>Smoking status</td>
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<td></td>
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<tr>
<td>Never</td>
<td>115</td>
<td>55.5</td>
<td>141</td>
<td>59.7</td>
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<td></td>
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<tr>
<td>Former</td>
<td>25</td>
<td>12.1</td>
<td>20</td>
<td>8.5</td>
<td>1.76</td>
<td>0.42</td>
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<td>Current</td>
<td>67</td>
<td>32.4</td>
<td>75</td>
<td>31.8</td>
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<tr>
<td>IR exposure history1</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>172</td>
<td>83.1</td>
<td>222</td>
<td>94.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ever</td>
<td>35</td>
<td>16.9</td>
<td>14</td>
<td>5.9</td>
<td>8.1</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>History of brain cancer in the first relatives</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>196</td>
<td>94.7</td>
<td>235</td>
<td>99.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>11</td>
<td>5.3</td>
<td>1</td>
<td>0.4</td>
<td>10.01</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Histological types2</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High grade glioma</td>
<td>111</td>
<td>53.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Medium grade glioma</td>
<td>47</td>
<td>22.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low grade glioma</td>
<td>49</td>
<td>23.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1Including 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. 2High 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 (1.07-2.47). Moreover, rs2276466 CC was associated with increased risk of glioma in dominant models, with ORs (95% CI) of 1.90 (1.05-3.44), and variant of rs1800067 G allele and rs2276466 G allele frequencies significantly associated with moderate enhanced risk of glioma when compared with CC genotype in codominant model, with OR (95% CI) of 2.63 (1.04-7.25). Moreover, rs2276466 GG was more likely to increase the risk of glioma when compared with A/A genotype in codominant model, with OR (95% CI) of 0.44 (0.19-0.98) (Table 4).

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

<table>
<thead>
<tr>
<th>dbSNP</th>
<th>Major/Minor allele</th>
<th>MAF Case</th>
<th>MAF Control</th>
<th>P for HWE OR(95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs3136038</td>
<td>C/T</td>
<td>35.2</td>
<td>34.3</td>
<td>0.03</td>
<td>1.05(0.79-1.40)</td>
</tr>
<tr>
<td>rs1799798</td>
<td>A/G</td>
<td>12.7</td>
<td>10.9</td>
<td>0.13</td>
<td>1.19(0.81-1.87)</td>
</tr>
<tr>
<td>rs1800067</td>
<td>G/A</td>
<td>17.9</td>
<td>12.7</td>
<td>0.08</td>
<td>1.49(1.02-2.21)</td>
</tr>
<tr>
<td>rs6498486</td>
<td>A/G</td>
<td>31.2</td>
<td>29.7</td>
<td>0.09</td>
<td>1.08(0.81-1.46)</td>
</tr>
<tr>
<td>rs2276465</td>
<td>G/C</td>
<td>32.1</td>
<td>29.5</td>
<td>0.14</td>
<td>1.15(0.85-1.56)</td>
</tr>
<tr>
<td>rs2276466</td>
<td>C/G</td>
<td>39.1</td>
<td>31.3</td>
<td>0.45</td>
<td>1.43(1.07-1.94)</td>
</tr>
</tbody>
</table>

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; Pan et al., 2013), 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 variants of rs1800067 and rs2276466 may be useful genetic susceptibility markers for glioma.
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.

Acknowledgements

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References

but fails to replicate associations from published candidate-

in the DNA repair gene XRCC1 and susceptibility to glioma in
a Han population in northeastern China: A case-control study.


CDKN2B and RTEL1 regions are associated with high-grade

nucleotide polymorphisms in ERCC4 and risk of squamous