Association of *ERCC1* and *XPF* polymorphisms with pediatric glioma susceptibility

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To the Editor: Glioma is a highly invasive and lethal heterogeneous disease caused by glial or precursor cell mutation. It accounts for about 50% of tumors in children and 80% of malignant tumors.[1] The nucleotide excision repair (NER) pathway is a process that involves the sequential assembly of many proteins, including excision repair cross complementation group 1 (ERCC1) and xeroderma pigmentosum complementation group F (XPF, also known as ERCC4). They play key roles in repairing DNA damages caused by ultraviolet light and helixdistorting adducts. The ERCC1–XPF forms a dimer to produce a multifunctional endonuclease essential for DNA repair.[2] Without DNA repair, DNA lesions aggregate and develop into glioma. The single nucleotide polymorphisms (SNPs) of genes involved in the DNA repair pathway may be potential targets for altering the risk of glioma. At present, the relationship between polymorphisms of these two genes and glioma has rarely been described.

Samples of glioma patients were collected from three regional hospitals in China while the controls were randomly selected from children visiting the hospital without any history of glioma. Cases and controls were recruited simultaneously based on age and sex as described in Supplementary Table 1, http://links.lww.com/CM9/B21. Informed consent was obtained from all participants and the trial was approved by the hospital's institutional review board (No. 2016021650).

The potential functional SNPs of chosen genes were selected by dbSNP database from http://www.ncbi.nlm.nih.gov/ and SNPinfo from http://snpinfo.niehs.nih.gov/. In brief, the identification criteria are to select candidate SNPs located in exons, 3′ untranslated region, 5′ flanking region, and 5′ UTR of the two genes, and to meet the requirements of low linkage and mutual imbalance. Finally, we found that four SNPs had potential functions, namely, three SNPs of *ERCC1,* which are rs2298881, rs11615, and rs3212986, and one SNP of *XPF* (rs2276466). Genomic DNA was extracted from peripheral blood using the QIAamp DNA blood kit (QIAGEN, Valencia, CA, USA). Then, we used ABI Q6 (Applied Biosystems, Foster City, CA, USA) for TaqMan genotyping of the above SNPs. The conditions of reactions were set as follows: pre-read stage at 60°C for 30 s; holding stage at 95°C for 10 min; repeated 45 cycles each of denaturation at 95°C for 15 s; and annealing and extension at 60°C for 1 min.

We used the goodness of fit χ^2-tests in the control group to estimate whether each SNP was consistent with Hardy–Weinberg equilibrium. The χ^2-test was used to analyze differences in demographic variables and distributions of allelic genotypes between cases and controls. Logistic regression analysis was also performed to calculate the age- and gender-adjusted odds ratio (ORs) values and 95% confidence interval (CIs) to quantify the degree of association. We also performed stratified analyses by age, sex, tumor subtypes, and clinical stage. All of the above

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Table 1: Association of *ERCC1* and *XPF* gene polymorphisms with glioma susceptibility.

Genotype	Cases ($n = 314$)	Controls ($n = 380$)	*P* value*	Crude OR (95% CI)	*P* value	Adjusted OR (95% CI)†	P value†
ERCC1 rs2298881 C > A (HWE = 0.353)							
CC	132 (42.04)	139 (36.58)		1.00		1.00	
CA	138 (43.95)	171 (45.00)		0.85 (0.61–1.18)	0.330	0.87 (0.63–1.21)	0.408
AA	44 (14.01)	70 (18.42)		0.66 (0.42–1.03)	0.070	0.67 (0.43–1.05)	0.081
Additive			0.069	0.82 (0.66–1.02)	0.069	0.83 (0.67–1.03)	0.086
Dominant	182 (57.96)	241 (63.42)	0.142	0.80 (0.59–1.08)	0.143	0.81 (0.60–1.11)	0.185
Recessive	270 (85.99)	310 (81.58)	0.119	0.72 (0.48–1.09)	0.120	0.72 (0.48–1.09)	0.122
ERCC1 rs11615 G > A (HWE = 0.034)							
GG	171 (54.46)	231 (60.79)		1.00		1.00	
GA	126 (40.13)	121 (31.84)		1.41 (1.02–1.93)	0.036	1.39 (1.01–1.91)	0.045
AA	17 (5.41)	28 (7.37)		0.82 (0.44–1.55)	0.540	0.81 (0.43–1.53)	0.515
Additive			0.352	1.12 (0.88–1.43)	0.352	1.11 (0.87–1.42)	0.400
Dominant	143 (45.54)	149 (39.21)	0.093	1.30 (0.96–1.76)	0.093	1.28 (0.94–1.73)	0.114
Recessive	297 (94.59)	352 (92.63)	0.298	0.72 (0.39–1.34)	0.300	0.71 (0.38–1.33)	0.290
ERCC*1* rs3212986 C > A (HWE = 0.474)							
CC	132 (42.04)	162 (42.63)		1.00		1.00	
CA	146 (46.50)	177 (46.58)		1.01 (0.74–1.39)	0.940	1.01 (0.73–1.39)	0.951
AA	36 (11.46)	41 (10.79)		1.08 (0.65–1.78)	0.771	1.08 (0.65–1.79)	0.761
Additive			0.801	1.03 (0.82–1.29)	0.801	1.03 (0.82–1.29)	0.798
Dominant	182 (57.96)	218 (57.37)	0.875	1.03 (0.76–1.39)	0.875	1.02 (0.76–1.39)	0.881
Recessive	278 (88.54)	339 (89.21)	0.778	1.07 (0.67–1.72)	0.778	1.08 (0.67–1.73)	0.763
XPF rs2276466 C > G (HWE = 0.633)							
CC	179 (57.74)	223 (58.68)		1.00		1.00	
CG	117 (37.74)	134 (35.26)		1.09 (0.79–1.49)	0.603	1.13 (0.82–1.56)	0.457
GG	14 (4.52)	23 (6.05)		0.76 (0.38–1.52)	0.434	0.75 (0.37–1.51)	0.420
Additive			0.897	0.98 (0.77–1.27)	0.897	1.00 (0.78–1.29)	1.000
Dominant	131 (42.26)	157 (41.32)	0.803	1.04 (0.77–1.41)	0.803	1.07 (0.79–1.46)	0.662
Recessive	296 (95.48)	357 (93.95)	0.373	0.73 (0.37–1.45)	0.375	0.72 (0.36–1.42)	0.343
Combined effect of risk genotypes‡							
0–1	61 (19.43)	96 (25.26)		1.00		1.00	
2–3	253 (80.57)	284 (74.74)	0.067	1.40 (0.98–2.02)	0.068	1.41 (0.98–2.02)	0.066

Data are presented as *n* (%). * χ^2 -test for genotype distributions between glioma patients and cancer-free controls. † Adjusted for age and gender. ‡ Risk genotypes were carriers with rs2298881 CC/CA, rs11615 GG/GA, rs3212986 CA/AA genotypes. CI: Confidence interval; ERCC1: Excision repair cross complementation group 1; HWE: Hardy–Weinberg equilibrium; OR: Odds ratio; XPF: Xeroderma pigmentosum complementation group F.

statistical data were analyzed on SAS v10.0 (SAS Institute, Inc., Cary, NC, USA), and a bilateral α value of 0.05 was set for the significance test.

The frequency distributions of selected demographic variables and clinical features between the two groups are described in detail in Supplementary Table 1, http://links.lww.com/CM9/B21. The effects of chosen SNPs on glioma susceptibility are illustrated explicitly in Table 1. Only *ERCC1* gene rs11615 was observed to up-regulate the glioma risk, which is probably a risk factor (GA *vs.* GG: adjusted OR = 1.39, 95% CI = 1.01–1.91, $P = 0.045$), whereas the other two SNPs (rs2298881 and rs3212986) in *ERCC1* and the rs2276466 in *XPF* provide no evidence to prove their relationship with the glioma susceptibility. Similar findings were not found in the combined analysis of risk genotypes.

The polymorphisms of the two genes were subject to stratified analysis to investigate whether different subgroups have impacts on glioma risk. As shown in Supplementary Table 2, http://links.lww.com/CM9/B21, rs2298881 was associated with a diminished risk of glioma in the strata of boys (adjusted OR = 0.64, 95% CI = 0.42–0.97, $P = 0.037$), astrocytic tumors (adjusted OR = 0.69, 95% CI = 0.49–0.97, $P = 0.034$), and clinical stage I (adjusted OR = 0.65,95% CI = 0.44–0.96, $P = 0.030$). On the other hand, rs11615 showed a significant enhancement in glioma risk in children with embryonal tumors (adjusted OR = 7.06, 95% CI = 1.51–33.07, $P = 0.013$) and clinical stage I (adjusted OR = 1.50, 95% CI = 1.02–2.20, $P = 0.037$). No further findings were found in the analysis of multiple risk genotypes. The *XPF* rs2276466 also did not support any association as illustrated in Supplementary Table 3, http://links.lww.com/CM9/B21.

In recent years, many epidemiological studies have revealed the association of DNA repair polymorphism with cancer susceptibility.[3] The genetic variations in *ERCC1/XPF* genes have been identified to impact some human genetic disorders.[4] Both ERCC1 and XPF are members of the XPF nuclease family (or MUS81 family). They interact with each other through the HhH_2 domain, thus forming a highly conserved heterodimer and exhibiting endonuclease activity. This complex works by cleaving the damaged DNA, creating two incisions that remove damaged oligonucleotides, and then inducing the concerted action of exonucleases, polymerases, and ligases to repair the gap.[5,6]

Variations in some alleles of *ERCC1* gene polymorphisms may attenuate the capacity of DNA repair, and these mutations are mainly concentrated in the 3′ UTR while there are several polymorphisms in the coding region of *XPF* that bring about possible genetic instability. Therefore, some *ERCC1/XPF* genes SNPs may have effects on glioma. Moreover, the chosen SNPs have potential functions. So, we hypothesized that these *ERCC1/XPF* genes SNPs are associated with glioma risk.

For the present study, we recruited Chinese Han children from multiple centers and conducted the first case-control study to explore the correlation. It turned out that except for *ERCC1* rs11615, no other genetic variation was associated with glioma risk. However, in some subgroups of stratified analysis, *ERCC1* rs2298881 and rs11615 were conferred with the potential to regulate glioma susceptibility.

Notably, the results of this study are different from those of some previous studies.[7] Consequently, the question of whether or not these *ERCC1/XPF* polymorphisms are related to the development of glioma is contradictory and needs further investigation. One possible reason for this could be the limited scale and heterogeneity among study populations, as well as the low frequency of high-risk genotype impacting on statistical inferences.

We believe that there are still some shortcomings in this experiment. First, the sample size is not enough and is only limited to three regions in southern China, which may influence the statistical deductions. Second, we lack functional experiments and need to understand how SNPs affect gliomas. Moreover, more attention should be paid to the interactions between environmental and genetic factors. Ultimately, only four SNPs were selected; further studies are needed to ascertain whether other polymorphisms of these two genes change the glioma risk. Besides, this is a study based on the Chinese Han population, and it may not be prudent to regard our results as being representative of the general population unless the ethnic or racial specificity in the present study can be addressed by suitable statistical methods.

In conclusion, we identified the relationship of some SNPs in *ERCC1* and *XPF* with glioma risk, providing reference evidence for further elucidating the genetic variation effect of the NER pathway gene. More studies are required to confirm our standpoint and to further reveal the underlying mechanisms of glioma.

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Conflicts of interest

None.

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