Short Communication

Association of an \textit{ERCC1} Polymorphism with Adult-Onset Glioma$^1$

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Abstract

Gliomas include several histologically distinct types of tumors whose molecular profiles suggest different etiologies. Because the \textit{ERCC1} protein is essential for nucleotide excision repair and influences genomic instability, polymorphisms in \textit{ERCC1} may play a role in human tumors. We determined the presence of the $A$ versus $C$ polymorphism at nucleotide 8092 of \textit{ERCC1} using a single-strand conformational polymorphism assay and DNA sequencing in adults with glioma and controls from a population-based study. Among 318 alleles from 159 controls, 27% (86) were $A$ and 73% were $C$. Prevalences of the $CC$ genotype were 51% (81 of 159), 48% (30 of 62), 63% (20 of 32), and 82% (23 of 28) for controls and subjects with glioblastoma multiforme, astrocytoma, and oligoastrocytoma, respectively (Fisher’s exact $P = 0.009$). The age-adjusted odds ratio for genotype $CC$ in all cases versus controls was 1.4 (95% confidence interval, 0.9–2.3), whereas that for subjects with glioblastoma multiforme, astrocytoma, and oligoastrocytoma, respectively (Fisher’s exact $P = 0.009$).

Introduction

An abnormal response to DNA damage resulting from endogenous or exogenous agents may contribute to genetic alterations leading to malignancy (1, 2). A wide variety of endogenous and exogenous agents cause various types of DNA damage. In the case of human brain tumors, the data on non-inherited factors from epidemiological studies designed to identify risk factors for brain tumor development are controversial. Although previous epidemiological studies indicate glioma was more common in men, in older people, and in people of white race (3), specific exposures or causative environmental agents have not been consistently identified, with the exception of therapeutic irradiation to the head (4–6), which being a relatively rare exposure, probably accounts for a relatively small proportion of cases.

Genetic factors that contribute to cancer susceptibility include both rare, highly penetrant, dominant mutations as well as more common genetic polymorphisms that influence individual response to environmental exposures. Genetic polymorphisms probably have an important role in determining cancer susceptibility and are the subject of intensive investigation for various cancer sites (7). Genetic polymorphisms are usually less penetrant than dominant mutations seen in retinoblastoma, Wilms’ tumor, and cancers of the Li-Fraumeni syndrome but are important to study because they have much higher prevalence and thus may have higher attributable risk. In our population based series of nearly 500 adults with glioma, only 4 patients ($<1\%$) had conditions known to genetically predispose to glioma (8). Given the important roles of genetic polymorphisms and DNA repair pathways in predisposition to malignancies, it is thus conceivable that polymorphisms in DNA repair genes that reduce activities of DNA repair pathways might predispose individuals to malignancies.

Here we investigated a recently discovered polymorphism in the \textit{3’}-untranslated region of \textit{ERCC1} (9), a subunit of the nucleotide excision repair complex. That no humans with a defect in \textit{ERCC1} have been identified and that there is no known amino acid sequence altering DNA polymorphism for this gene indicate tight control through evolution and imply essential functions for viability. \textit{ERCC1}, as well as XPA protein (xeroderma pigmentosum complementation group A), have been shown to be absolutely required for the incision step of nucleotide excision repair (10). Cells from \textit{ERCC1}-deficient mice show increased genomic instability and a reduced frequency of S-phase-dependent illegitimate chromosome exchange and signs of premature aging in addition to a repair-deficient phenotype (11). Therefore, \textit{ERCC1} may be important in repairing DNA damage (removal of DNA adducts and rejoining of double-strand DNA breaks caused by X-ray irradiation) that may be important for the development of brain tumors. Furthermore, among xeroderma pigmentosum patients $<40$ years of age with internal cancer, there was a dispropor-

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Table 1  Comparison of the study group included in ERCC1 genotype analysis with the overall study population of brain tumor cases and controls in the San Francisco Bay Area Adult Glioma Study, 1991–1995

<table>
<thead>
<tr>
<th>Factor</th>
<th>Total study population</th>
<th>ERCC1 study subsetab</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases (n = 476)</td>
<td>Controls (n = 462)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>204 (43)</td>
<td>209 (45)</td>
</tr>
<tr>
<td>Male</td>
<td>272 (57)</td>
<td>253 (55)</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>54.2 ± 0.8</td>
<td>53.7 ± 0.8</td>
</tr>
<tr>
<td>Diagnosis by cell typec</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glioblastoma multiforme</td>
<td>281 (67)</td>
<td>62 (51)</td>
</tr>
<tr>
<td>Astrocytoma</td>
<td>89 (21)</td>
<td>32 (26)</td>
</tr>
<tr>
<td>Oligoastrocytoma</td>
<td>47 (11)</td>
<td>28 (23)</td>
</tr>
<tr>
<td>Race (white)</td>
<td>402 (84)</td>
<td>396 (86)</td>
</tr>
</tbody>
</table>

ab Data are expressed as No. (%) and mean ± SE.
bc Whites only.
d Percentages shown are percent of 417 tumors with astrocytic component.

tionate representation of malignant neoplasm of the brain and oral cavity compared with United States whites <40 years of age (12), supporting the idea that excision repair could be important in neuro-oncogenesis.

In this report, we examine an A/C polymorphism at 8092 of ERCC1 (GenBank accession no. M63796) described by Shen et al. (9), which may affect mRNA stability, for its possible association with adult glioma.

Materials and Methods

Subjects. Cases and controls for this study were drawn from the San Francisco Bay Area adult glioma study discussed in detail elsewhere (8). We ascertained 492 incident glioma cases (ages >20 years) from August 1991 to April 1994 in six San Francisco Bay Area counties through the Northern California Cancer Center’s rapid case ascertainment service. Uniform neuropathology review indicated that four cases were not glioma and that specimens could not be reviewed for 12 subjects. Thus, the parent study includes 476 cases. Four hundred sixty-two controls were contacted through a random digit dialing technique and were frequency-matched for gender, ethnicity, and age (8). We began collecting blood specimens part way through the study (13, 14), and we obtained blood from 187 cases with pathology review and 171 controls. Only white cases and controls were included in genotyping ERCC1 because of ethnic differences in the distribution of polymorphisms (15–17) and because 84% of cases were white. The parent study consisted of 164 white controls and 161 white cases, of whom only the 129 with an astrocytic component, i.e., diagnoses of glioblastoma, astrocytoma (astrocytoma and anaplastic astrocytoma), or oligoastrocytoma, were further considered. Of these subjects, DNA was insufficient for PCR amplification for two subjects with astrocytoma, five with glioblastoma, and five controls. Thus, 159 controls and 122 cases of white ethnicity were included in the present studies.

Genotyping of ERCC1 Polymorphism. PCR-SSCP3 assay and DNA sequencing were used to determine the frequency of the polymorphisms. The use of SSCP as a method for genotyping polymorphisms has been described (15), and we also have used this method of genotyping successfully in other polymorphic markers we are studying in the laboratory. Briefly, oligonucleotide primers 5′-TGAAGCCAATTCAGCCACT-3′ and 5′-TGTTCCTCAGTTTCCCCG-3′ for PCR amplification of 255-bp fragments were synthesized by Operon Technology Inc. (Alameda, CA). PCR products were generated in a 30-µl reaction mixture, including 50 ng of DNA, 20 µM deoxyribonucleotide triphosphates, 10 mM Tris-HCl (pH 9.0), 1.5 mM MgCl2, 0.1% Triton X-100, 10 pmol of each primer, 1 unit of Taq (Perkin-Elmer Cetus, Norwalk, CT), and 0.2 µCi of [32P]dCTP (DuPont New England Nuclear, Boston, MA). The PCR reaction was carried out using 35 cycles (94°C for 30 s, 60°C for 30 s, and 72°C for 1 min) on a Perkin-Elmer 9600 thermal cycler. Ten µl of PCR product were diluted with 90 µl of 0.1% SDS-10 mM EDTA buffer. The diluted sample was then mixed 1:1 with gel-loading buffer solution from United States Biochemical Corp. (Cleveland, OH) and heated at 94°C for 4 min. The sample was kept on ice and loaded immediately onto 6% nondenatured polyacrylamide gel supplemented with 10% glycerol. The gel was run at room temperature for 20 h and exposed for 16 h for autoradiographic detection of bands. Direct sequencing of PCR fragments was performed on representative DNA samples of different migration patterns on SSCP gel to determine the corresponding DNA sequences using the dsDNA cycle sequencing system from Life Technologies (Gaithersburg, MD).

Statistical Analyses. ORs were computed for dichotomous factors, and means or medians were compared for continuous data. Then, 95% CIs on ORs or mean differences were used to assess precision of the estimates. We used a Fisher’s exact test to compare the prevalence of CC versus AC or AA genotypes among controls and the three histological types, glioblastoma multiforme, astrocytoma, and oligoastrocytoma (18). Logistic regression was used to estimate unadjusted and age-adjusted ORs for having the CC genotype in each histological category versus controls. Wilcoxon tests were used to compare median ages of diagnosis among those with and without the CC genotype regardless of histological type and within each histological type. Statistical analyses were conducted with SAS software for personal computers (19).

Results

We compared the prevalence of the ERCC1 8092 polymorphism in 122 Caucasian adult glioma patients with 159 controls. Table 1 compares the demographic characteristics and tumor histology of cases in this genotyping study group with

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3 The abbreviations used are: SSCP, single-strand conformational polymorphism; OR, odds ratio; CI, confidence interval; ASE-1, antisense of ERCC1; CAST, CD3e-associated signal transducer.
for glioma cases with age of onset of disease, we compared median ages at diagnosis patients with oligoastrocytoma versus CC trocytoma versus P 0.009; Fisher’s exact test of the 4 3 contingency table) and 20 years younger than those with glioblastoma, and for oligoastrocytoma patients, it was ~20 years younger than for those with glioblastoma. Thus, our results indicated that the CC genotype appears to be associated with oligoastrocytoma, the histological subtype of glioma (of the three considered here) with the youngest median age at onset.

**Discussion**

Our main finding was that the ERCC1 nucleotide 8092 genotype CC was statistically significantly more common among patients with oligoastrocytoma compared with controls. The CC genotype also was somewhat more common in patients with astrocytoma compared with controls, but the genotype frequency was similar in patients with glioblastoma and controls. The potential involvement of the constitutive ERCC1 variant with histological subtypes of glioma is consistent with multiple pathogenic mechanisms of these tumors. For example, genetic alteration models from previous molecular genetic studies of tumor DNA from glioma patients indicated the existence of at least two types of glioblastoma multiforme (20). It is thought that one type of glioblastoma multiforme results from progression from lower grades of astrocytomas, whereas the other type is *de novo* in nature. Because this series of adult glioma cases was collected through reports of only newly diagnosed cases, glioblastoma tumors of the *de novo* type likely predominate in this series. Such *de novo* tumors would be likely to have different genetic alterations than the astrocytomas from which the other type of glioblastoma multiforme may be derived. If our hypothesis is correct, we might predict that subjects with glioblastoma multiforme that progressed from lower grade tumors would have a higher prevalence of the ERCC1 CC genotype than the present series of glioblastoma multiforme cases.

The other interesting finding was that glioma patients with the CC genotype had an earlier median age at diagnosis than glioma patients with AA or AC genotypes. Although interpretation of these results is complicated by the differing median ages at onset for the different histological types of gliomas, the CC genotype is most frequent in those subjects with oligoastrocytoma, the histological type with the earliest median age at onset. The pattern of differences in age at diagnosis in this study is similar to that expected based on population figures. For example, the Central Brain Tumor Registry of the United States reports mean ages of diagnosis for glioblastoma, anaplastic astrocytoma, diffuse astrocytoma, and oligoastrocytoma for the period 1990–1994 of 62, 50, 47, and 40 years, respectively (21). Because survival from glioma decreases with age, blood samples were obtained somewhat disproportionately from younger patients; thus, with this sample, we cannot completely rule out the possibility of a role for the ERCC1 CC genotype in progression or survival versus etiology. It would be of interest to see if this polymorphism is associated with early onset in other cancer sites.

DNA damage responses play a central role in neoplastic transformation and are involved in both mechanisms identified as potential risk factors for brain tumors. ERCC1 may be of particular importance because it may be involved in both removal of DNA adducts caused by nitroso-compounds and rejoicing of double-strand DNA breaks caused by X-ray irradiation that are important for development of brain tumors. The data presented in this study is the first to show an association of a polymorphism in ERCC1 with the risk of brain tumor. Despite the relatively small sample sizes, the highly statistically signifi-

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**Table 2** Frequencies of ERCC1 genotypes in white brain tumor patients and controls, stratified by tumor histopathology in the San Francisco Bay Area Adult Glioma Study, 1991–1995

<table>
<thead>
<tr>
<th>Group</th>
<th>ERCC1 Genotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AA</td>
</tr>
<tr>
<td>Control (n = 159)</td>
<td>8 (5)</td>
</tr>
<tr>
<td>All cases (n = 122)</td>
<td>6 (5)</td>
</tr>
<tr>
<td>Glioblastoma multiforme (n = 62)</td>
<td>5 (8)</td>
</tr>
<tr>
<td>Astrocytoma (n = 32)</td>
<td>1 (3)</td>
</tr>
<tr>
<td>Oligoastrocytoma (n = 28)</td>
<td>0</td>
</tr>
</tbody>
</table>

* Data are expressed as No. (%).  
* P < 0.05 by Fisher’s exact test that the frequencies of genotype CC are the same in controls and in the three patient groups.
Table 3  Numbers and median ages of ERCC1 genotypes AA/AC versus CC in white brain tumor patients and controls, stratified by tumor histopathology in the San Francisco Bay Area Adult Glioma Study, 1991–1995

<table>
<thead>
<tr>
<th>Group</th>
<th>Genotypes and median ages</th>
<th>ORs (95% CI)</th>
<th>Age-adjusted OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of AA/AC (median age)</td>
<td>No. of CC (median age)</td>
<td></td>
</tr>
<tr>
<td>Control (n = 159)</td>
<td>78 (51 yr)</td>
<td>81 (55 yr)</td>
<td>1.0*</td>
</tr>
<tr>
<td>All cases (n = 122)</td>
<td>49 (54 yr)</td>
<td>73 (46 yr)</td>
<td>1.4 (0.9–2.3)</td>
</tr>
<tr>
<td>Glioblastoma (n = 62)</td>
<td>32 (58 yr)</td>
<td>30 (58 yr)</td>
<td>0.9 (0.5–1.6)</td>
</tr>
<tr>
<td>Astrocytoma (n = 32)</td>
<td>12 (43 yr)</td>
<td>20 (41 yr)</td>
<td>1.6 (0.7–3.5)</td>
</tr>
<tr>
<td>Oligoastrocytoma (n = 28)</td>
<td>5 (39 yr)</td>
<td>23 (33 yr)</td>
<td>4.4 (1.6–12.2)</td>
</tr>
</tbody>
</table>

* Age is at diagnosis for cases and at interview for controls.

References


11. Melton, D. W., Ketchen, A. M., Núñez, F., Bonatti-Abbondandolo, S., Abbondandolo, A., Squires, S., and Johnson, R. T. Cells from ERCC1-deficient mice show increased genome instability and a reduced frequency of 5-phase-


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