Associations between Polymorphisms in DNA Repair Genes and Glioblastoma

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Abstract

A pooled analysis was conducted to examine the association between select variants in DNA repair genes and glioblastoma multiforme, the most common and deadliest form of adult brain tumors. Genetic data for ~1,000 glioblastoma multiforme cases and 2,000 controls were combined from four centers in the United States that have conducted case-control studies on adult glioblastoma multiforme, including the National Cancer Institute, the National Institute for Occupational Safety and Health, the University of Texas M. D. Anderson Cancer Center, and the University of California at San Francisco. Twelve DNA repair single-nucleotide polymorphisms were selected for investigation in the pilot collaborative project. The *C* allele of the *PARP1* rs1136410 variant was associated with a 20% reduction in risk for glioblastoma multiforme (odds ratio$_{CT\ or\ CC}$, 0.80; 95% confidence interval, 0.67-0.95). A 44% increase in risk for glioblastoma multiforme was found for individuals homozygous for the *G* allele of the *PRKDC* rs7003908 variant (odds ratio$_{GG}$, 1.44; 95% confidence interval, 1.13-1.84); there was a statistically significant trend (P = 0.009) with increasing number of *G* alleles. A significant, protective effect was found when three single-nucleotide polymorphisms (*ERCC2* rs13181, *ERCC1* rs3212986, and *GLTSCR1* rs1035938) located near each other on chromosome 19 were modeled as a haplotype. The most common haplotype (AGC) was associated with a 23% reduction in risk (P = 0.03) compared with all other haplotypes combined. Few studies have reported on the associations between variants in DNA repair genes and brain tumors, and few specifically have examined their impact on glioblastoma multiforme. Our results suggest that common variation in DNA repair genes may be associated with risk for glioblastoma multiforme. (Cancer Epidemiol Biomarkers Prev 2009;18(4):1118–26)

Introduction

Glioblastoma multiforme is the most common primary brain tumor in adults (1); it is a highly malignant grade 4 glioma that is rapidly fatal, with a 1-year and 5-year survival of 30% and 3%, respectively (2). The causes of glioblastoma multiforme are largely unknown, with no single risk factor identified that explains a large proportion of cases. Although ionizing radiation is a clearly established environmental cause of brain tumors, high dose exposure to ionizing radiation is rare and therefore unlikely to explain many new cases of brain tumors (3). Other residential, occupational, and lifestyle factors have not been consistently associated with brain tumor risk in epidemiologic studies (4). Family history of glioma and several genetic syndromes, including neurofibromatosis types I and II, Li-Fraumeni syndrome, Gorlin syndrome, and Turcot syndrome, provide evidence of a genetic predisposition to brain cancer but are likely to account for only a small proportion of cases (5). The search for genetic and preventable environmental risk factors of glioblastoma multiforme therefore remains of critical importance.

Deficiencies in DNA repair pathways have been associated with cancer risk, cancer-related syndromes (6), and sensitivity to chemotherapeutic agents (7). DNA repair enzymes continuously monitor for damaged nucleotide residues generated by exposure to carcinogens and cytotoxic compounds (8). All tissues, including brain, may incur damage because of mutagens resulting from normal cellular processes or from environmental exposures. A high proportion of mutations are caused by weak mutagens produced in the body, including reactive oxygen species and other cellular metabolites. These metabolites result in slow turnover of DNA, even in cells that normally do not proliferate (8, 9). Exogenous agents that have been considered potential neurocarcinogens

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such as pesticides, *N*-nitroso compounds, and tobacco smoke may also contribute to the accumulation of chromosomal mutations and structural breaks.

Damage resulting from endogenous or exogenous exposure may be corrected by enzymes coded by one or more DNA repair pathways. Each pathway is recognized for efficient repair of specific types of DNA damage. Base excision repair is a multistep process for the removal of small base adducts such as those produced by methylation or oxidation (10). Nucleotide excision repair corrects UV-induced lesions, intrastrand cross-links, and bulky adducts following exposure to a range of environmental chemicals such as polycyclic aromatic hydrocarbons, aromatic amines, benzopyrenes, and some types of *N*-nitroso compounds (11-13). Direct repair is another mechanism that acts to reverse rather than excise DNA damage, typically involving methyl and other small alkyl groups (8). More severe double-strand breaks may occur following exposure to ionizing radiation or to products of cellular processes resulting in hydrolysis, oxidation, or methylation of DNA. Nonhomologous end joining is believed to be the primary repair pathway for most double-strand breaks, including those resulting from ionizing radiation (14). Homologous recombination is an alternative repair mechanism for double-strand breaks; however, the process is more closely tied with chromatin replication. Genetic variation may alter the function of DNA repair proteins and therefore influence glioma risk.

Few studies have reported on the associations between variants in DNA repair genes and brain tumors, and few specifically have examined their impact on glioblastoma multiforme. In the current analysis, we examined the association between select variants in DNA repair genes and risk for glioblastoma multiforme. We combined genetic data using DNA specimens from four centers in the United States that have conducted studies on gliomas, including the National Cancer Institute (NCI), the National Institute for Occupational Safety and Health (NIOSH), the University of Texas M. D. Anderson (MDA) Cancer Center, and the University of California at San Francisco (UCSF). We selected 12 putative functional single-nucleotide polymorphisms in DNA repair genes that were previously investigated at one or more of the four study centers and then completed genotyping at the remaining centers for each of the selected DNA repair variants. The pooled analysis includes genotyping data from ~1,000 glioblastoma multiforme cases and 2,000 controls.

Materials and Methods

Study Population. Data for these analyses were assembled from four existing case-control studies of brain tumors in the United States: (*a*) the Multicenter Study of Environment and Health by investigators at the NCI; (*b*) the Upper Midwest Health Study by investigators at the NIOSH of the Centers for Disease Control and Prevention; (*c*) the Family Health Study by investigators at The University of Texas MDA Cancer Center, and (*d*) the Genetic and Molecular Epidemiology of Adult Glioma Study by investigators at the UCSF. The collaborative effort was established through meetings organized by the Brain Tumor Epidemiology Consortium, an international organization established in 2003 to develop multicenter and interdisciplinary collaborations that will lead to a better understanding of the etiology, outcomes, and prevention of brain tumors.[7]

Twelve single-nucleotide polymorphisms in 11 candidate DNA repair genes were selected for testing in the pilot study (Table 1). These pathways were considered relevant to the types of DNA damage that could result from exposure to potential neurocarcinogens (e.g., ionizing radiation, pesticides, polycyclic aromatic hydrocarbons, *N*-nitroso compounds) or products of cellular metabolic processes. We selected single-nucleotide polymorphisms in DNA repair genes that have previously been investigated as glioma risk factors at one or more of the four study centers and were believed to be functional or have been associated with risk for glioma in the literature. All single-nucleotide polymorphisms had minor allele frequencies of >0.1. Five single-nucleotide polymorphisms were selected from the base excision repair pathway (*OGG1* rs1052133, *APEX1* rs1130409, *XRCC1* rs25487, *XRCC1* rs1799782, *PARP* rs1136410); five single-nucleotide polymorphisms were selected from the nucleotide excision repair pathway (*ERCC2* rs13181, *RAD23B* rs1805329, *ERCC5* rs17655, *GLTSCR1* rs1035938, *ERCC1* rs3212986); one single-nucleotide polymorphism was selected from the direct repair pathway (*MGMT* rs12917); and one single-nucleotide polymorphism from the nonhomologous end–joining pathway (*PRKDC* rs7003908).

Existing DNA samples and demographic data for 1,015 cases of glioblastoma multiforme and 1,994 controls from the four study centers were identified for inclusion in the pooled analyses. Cases for the combined analyses include adults 18 y of age and older at the time of diagnosis with histologically confirmed primary glioblastoma (International Classification of Diseases for Oncology code 9440). Case and control selection procedures differed by study center. Methods for each of the studies have been published previously (15-18). Briefly, study procedures were as follows.

1. The Multicenter Study of Environment and Health (NCI): cases were identified from three hospitals in Phoenix, Arizona; Boston, Massachusetts; and Pittsburgh, Pennsylvania, from 1994 to 1998. Hospital-based controls were identified from non-cancer-related admissions (17, 19).
2. The Upper Midwest Health Study (NIOSH): cases were identified from residents of eligible nonmetropolitan counties in Iowa, Michigan, Minnesota, and Wisconsin, who were diagnosed with a glioma from 1995 to 1997. Population-based controls were randomly selected from state driver's license and Medicare files from the same counties. Cases were required to have a state driver's license, have an identity card, or be enrolled in Medicare to maintain comparability with controls (20).
3. The Harris County Case-Control Study (University of Texas MDA Cancer Center): cases were identified through physician or clinic referrals at hospitals where brain tumor patients are diagnosed in Harris

[7] Brain Tumor Epidemiology Consortium. http://epi.grants.cancer.gov/btec/.

Table 1. Candidate DNA repair pathway genes, Glioblastoma Collaborative Group, 2008

Pathway	Gene name	Gene	SNP ID	SNP	Base change	Chr
Direct	Methyl-guanine methyltransferase	*MGMT*	rs12917	Leu84Phe	C/T	10q26.3
BER	8-Hydroxyguanine DNA glycosylase	*OGG1*	rs1052133	Ser326Cys	C/G	3p26.2
BER	Apurinic endonuclease	*APEX1*	rs1130409	Asp148Glu	T/G	14q11.2
BER	X-ray repair, complementing defective, 1	*XRCC1*	rs1799782	Arg194Trp	G/A	19q13.2
BER	X-ray repair, complementing defective, 1	*XRCC1*	rs25487	Arg399Gly	C/T	19q13.2
BER	ADP-ribosyltransferase	*PARP1*	rs1136410	Val762Ala	T/C	1q41
NER	Excision repair, complementing defective, 2	*ERCC2*	rs13181	Lys751Gln	A/C	19q13.3
NER	RAD23	*RAD23B*	rs1805329	Ala249Val	C/T	9q31.2
NER	Excision repair, complementing defective, 5	*ERCC5*	rs17655	His1104Asp	G/C	13q22
NER	Glioma tumor suppressor candidate region	*GLTSCR1*	rs1035938	Ser387Ser	C/T	19q13.3
NER	Excision repair, complementing defective, 1	*ERCC1*	rs3212986	C8092A	C/A	19q13.2
NHEJ	DNA-dependent protein kinase	*PRKDC*	rs7003908	6721G>T	G/T	8q11

Abbreviations: SNP, single-nucleotide polymorphism; ID, identification; Chr, chromosome; BER, base excision repair; NER, nucleotide excision repair; NHEJ, nonhomologous end–joining.

County, Texas. Population-based controls were identified by random-digit dialing. Consenting participants provided blood and/or buccal specimens and information in an in-person or telephone interview.

4. The Genetic and Molecular Epidemiology of Adult Glioma Study (UCSF): newly diagnosed cases of glioblastoma multiforme residing in six San Francisco Bay Area counties (San Francisco, Alameda, Contra Costa, Santa Clara, San Mateo, Marin) were ascertained between 1991 and 1994, 1997 and 1999, and 2001 and 2004 through the Northern California Cancer Center's Rapid Case Ascertainment program (21, 22). In addition to population-based recruitment, newly diagnosed glioblastoma patients attending the UCSF Neuro-oncology Clinic from 2002 to 2006 were asked to participate regardless of residence in the San Francisco Bay Area. Controls were identified through random-digit dialing (22). Consenting participants provided blood and/or buccal specimens and information in an in-person or telephone interview. Only White participants with at least five aliquots of blood were eligible for this present study to conduct the additional genotyping; thus, the numbers of participants are lower than the totals from other articles published from this group.

In-person interviews were used to collect demographic, lifestyle, and environmental exposure histories at each of the four centers, as well as blood samples for DNA extraction and genotyping. Informed consent was obtained for participants at each study site. Individual study protocols were approved by the appropriate institutional review board at each participating center and at the University of Southern California for the pooled analyses.

At all sites, controls were frequency matched to cases on age at diagnosis and gender; controls from NCI, MDA, and UCSF were additionally matched on race/ethnicity. The pooled genetic analyses were restricted to adults who described themselves as non-Hispanic White; other racial/ethnic groups were excluded because there were too few individuals in these categories to analyze the data separately with sufficient statistical power.

Laboratory Methods. DNA was extracted from blood samples and genotyped by individual laboratories used by each of the study centers. For NCI and NIOSH samples, DNA was extracted from peripheral WBCs using a phenol-chloroform method (23) and genotyped for the DNA repair single-nucleotide polymorphisms using custom-designed Taqman genotyping assays (Applied Biosystems) at the NCI Core Genotyping Facility (Gaithersburg, MD). At UCSF, DNA was extracted from heparinized whole blood using Qiagen column purification or Autogen DNA. Genotyping for most samples was completed using the same custom-designed Taqman assays (Applied Biosystems) used by the NCI laboratory. For 112 UCSF cases and 114 controls, the *RAD23B, ERCC5, APEX1*, and *PARP1* genotype results were obtained from existing pilot data determined using the ParAllele 10,000 nonsynonymous coding single-nucleotide polymorphism assay panel (24-26). At MDA, genomic DNA was extracted from peripheral blood lymphocytes using the Qiagen Blood Kit. Genotyping was done using the Sequenom MassARRAY iPLEX platform.[8] MassARRAY Workstation version 3.3 software was used to process and analyze iPLEX SpectroCHIP bioarrays.

For quality control purposes, 10% replicate samples, negative water controls, and 15 DNA standards with known genotypes (Coriell Biorepository) were included in the genotyping assays at the three laboratories. At the NCI laboratory, an additional 85 DNA standard controls (100 total; Coriell Biorepository) were included in the pilot genotyping plates.

Statistical Analyses. The distribution of allele frequencies for cases and controls was examined overall and by study center. Pearson χ^2 tests were used to assess the consistency of allele frequencies with Hardy-Weinberg equilibrium among the controls. Summary and stratum-specific odds ratios and 95% confidence intervals (95% CI) were calculated using unconditional logistic regression to evaluate the association between each DNA repair single-nucleotide polymorphism and risk for glioblastoma for all samples and by age (<50, 50+ y at diagnosis) and study center adjusted for age at diagnosis (or reference age for controls), gender, and/or study center where appropriate. Adjustment for other potential glioblastoma multiforme risk factors, including education and family history of cancer or brain cancer,

[8] http://www.sequenom.com/seq.genotyping.html

was completed; however, these variables were not included in the final models because they did not substantially change the findings. Results are presented by genotype with the more common homozygote as the reference group. Tests for trends were calculated using genotype as an ordinal variable in the regression models. To further assess the significance of single-nucleotide polymorphism association results, we conducted permutation testing. In brief, 10,000 data sets were generated through Monte Carlo sampling. Each of these data sets was analyzed in a manner identical to that of the original unpermuted data. The proportion of permuted replicates for which the smallest P value was less than the P value observed from the original data was then used as the overall association test of significance.

Potential gene-gene interactions were investigated using a focused interaction testing framework (27). Interactions were evaluated considering all 12 single-nucleotide polymorphisms and after restricting to single-nucleotide polymorphisms in common pathways (e.g., base excision repair, nucleotide excision repair). Likelihood-ratio tests were done in stages that increase in the order of interaction considered. Joint tests of main effects and interactions were done, conditional on significant lower-order effects. A reduction in the number of tests done is achieved by prescreening gene combinations with a goodness-of-fit χ^2 statistical that depends on association among candidate genes in the pooled case-control group. Multiple testing is accounted for by controlling false-discovery rates.

Haplotype analyses were done using three nucleotide excision repair pathway single-nucleotide polymorphisms in genes located on the long arm of chromosome 19 (*ERCC1* rs3212986, *ERCC2* rs13181, *GLTSCR1* rs1035938). These genes were the only tested genes residing in the same common chromosomal region with each other on 19q13; the *XRCC1* single-nucleotide polymorphisms were not included because they are in the base excision repair pathway and not in close proximity of the nucleotide excision repair genes. Haplotype frequency estimates were constructed from genotype data using the expectation-maximization algorithm (28). Haplotype frequencies were estimated for each individual, conditional on the genotype of the individual. The estimates were used within logistic regression models to estimate odds ratios and 95% CIs.

Calculations were done using PROC LOGISTIC (SAS V 9.1) and PROC HAPLOTYPE from SAS/Genetics (SAS Institute).

Results

A total of 1,015 glioblastoma multiforme cases and 1,994 controls were genotyped for 12 single-nucleotide polymorphisms in the DNA repair candidate pathways. Of these, 213 were contributed by MDA, 171 from NCI, 139 from NIOSH, and 492 from UCSF (Table 2). In the pooled data, most cases were male (Table 2) and 66% of the cases had some college training or were a college graduate (data not shown). Cases were more likely to be male than the control group (61% versus 51%), but were similar to controls with respect to education level. The mean age of cases (56 years) at diagnosis was slightly older than the reference age for controls (54 years).

Table 2. Characteristics of glioblastoma cases and controls, Glioblastoma Collaborative Group, 2008

	Case		Control	
	No.	(%)	No.	(%)
All sites	1,015		1,994	
MDA	213	(20.9)	365	(18.3)
NCI	171	(16.8)	489	(24.5)
NIOSH	139	(13.7)	453	(22.7)
UCSF	492	(48.5)	687	(34.5)
Gender				
Male	619	(61.0)	1,020	(51.1)
Female	396	(39.0)	974	(48.9)
Age*± SD	56.3 ± 12.6		53.6 ± 15.3	

*Age at diagnosis for cases and reference age for controls.

All single-nucleotide polymorphisms were consistent with Hardy-Weinberg equilibrium for controls at each study center. Data from the four study centers were in complete agreement with known genotypes for the 15 Coriell DNA standards. Genotypes for NCI and NIOSH samples also were in agreement with the 85 additional samples tested at the NCI laboratory. Genotyping completion was ≥96% for the 12 single-nucleotide polymorphisms. Concordance among duplicate samples was ≥97% for samples missing a call or with mismatched calls and ≥99% for duplicate samples with calls. The call rates for individual single-nucleotide polymorphisms ranged from 96% (GLTSCR, ERCC1) to 99% (ERCC2). Numbers of individuals genotyped for each single-nucleotide polymorphism are shown in Table 3.

Among the five base excision repair genes, only the *PARP1* single-nucleotide polymorphism (rs1136410) was significantly associated with risk for glioblastoma multiforme. No significant associations were found for the remaining four base excision repair single-nucleotide polymorphisms (*APEX1* rs1130409, *OGG1* rs1052133, *XRCC1* rs25487, *XRCC1* rs1799782) or for the single direct-repair single-nucleotide polymorphism (*MGMT* rs12917; Table 3). Participants with one or two copies of the *PARP1 C* allele were at lower risk for a glioblastoma multiforme than participants homozygous for the *T* allele (odds ratio$_{CT\ or\ CC}$, 0.80; 95% CI, 0.67-0.95). Risk was similar for individuals heterozygote or homozygous for the *C* allele (odds ratio$_{CT}$, 0.79; 95% CI, 0.67-0.95; odds ratio$_{CC}$, 0.83; 95% CI, 0.51-1.38; P_{trend} = 0.016; Table 3). The reduction in risk associated with the *C* allele was consistent across study centers ($P_{interaction}$ = 0.81; data not shown), and the *C* allele frequency of 17% is consistent with the minor allele frequency reported on the National Center for Biotechnology Information website for Caucasian populations.[9] We found a significant trend in risk by genotype that was present for individuals 50 years of age and older but not for individuals <50 years of age at diagnosis (Supplementary Table 1).

We found a significant trend of increasing risk with the *G* allele of the intronic *PRKDC* variant (rs7003908; P_{trend} = 0.009). Individuals who were homozygous for the *G* allele were 44% more likely to be diagnosed with a glioblastoma multiforme than individuals with the

[9] http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?type=rs&rs=1136410

Table 3. Odds ratios and 95% CI for DNA repair single-nucleotide polymorphisms and risk for glioblastoma for all centers combined, Glioblastoma Collaborative Group, 2008

Gene SNP	Genotype	Cases* (%)	Controls* (%)	OR†	95% CI
BER					
APEX1 rs1130409					
	TT	265 (26.8)	535 (27.8)	Ref	
	GT	510 (51.6)	945 (49.1)	1.09	0.90-1.31
	GG	213 (21.6)	446 (23.1)	1.00	0.80-1.25
				P_{trend} = 0.96	
OGG1 rs1052133					
	CC	596 (59.9)	1,150 (59.3)	Ref	
	CG	347 (34.9)	676 (34.9)	0.96	0.81-1.13
	GG	52 (5.2)	112 (5.8)	0.88	0.62-1.25
				P_{trend} = 0.43	
PARP1 rs1136410					
	TT	713 (72.2)	1,303 (67.3)	Ref	
	CT	251 (25.4)	575 (29.7)	0.79	0.67-0.95
	CC	23 (2.3)	57 (3.0)	0.83	0.51-1.38
				P_{trend} = 0.02	
XRCC1 rs25487					
	GG	397 (39.6)	844 (42.8)	Ref	
	AG	461 (46.0)	865 (43.9)	1.12	0.95-1.33
	AA	145 (14.5)	262 (13.3)	1.23	0.96-1.57
				P_{trend} = 0.07	
XRCC1 rs1799782					
	CC	842 (87.5)	1,664 (86.6)	Ref	
	CT	117 (12.2)	252 (13.1)	0.89	0.70-1.14
	TT	3 (0.3)	6 (0.3)	0.84	0.21-3.45
				P_{trend} = 0.35	
Direct repair					
MGMT rs12917					
	CC	774 (77.6)	1,480 (75.2)	Ref	
	CT	204 (20.4)	453 (23.0)	0.89	0.73-1.07
	TT	20 (2.0)	35 (1.8)	1.24	0.70-2.20
				P_{trend} = 0.50	
NHEJ					
PRKDC rs7003908					
	TT	389 (41.8)	811 (42.3)	Ref	
	GT	397 (42.6)	875 (45.7)	1.07	0.90-1.27
	GG	145 (15.6)	230 (12.0)	1.44	1.13-1.84
				P_{trend} = 0.009	
NER					
ERCC1 rs3212986					
	CC	557 (57.0)	1,087 (56.6)	Ref	
	CA	361 (36.9)	728 (37.9)	0.99	0.84-1.17
	AA	59 (6.0)	105 (5.5)	1.09	0.77-1.54
				P_{trend} = 0.84	
ERCC2 rs13181					
	AA	376 (37.6)	823 (41.8)	Ref	
	AC	480 (48.0)	891 (45.2)	1.16	0.98-1.37
	CC	143 (14.3)	256 (13.0)	1.19	0.93-1.52
				P_{trend} = 0.08	
ERCC5 rs17655					
	GG	499 (49.7)	989 (50.5)	Ref	
	CG	348 (34.7)	657 (33.6)	0.98	0.82-1.18
	CC	157 (15.6)	311 (15.9)	0.81	0.60-1.08
				P_{trend} = 0.25	
GLTSCR1 rs1035938					
	CC	503 (51.2)	1,017 (53.2)	Ref	
	CT	395 (40.2)	761 (39.8)	1.06	0.90-1.25
	TT	84 (8.6)	135 (7.1)	1.24	0.92-1.67
				P_{trend} = 0.18	
RAD23B rs1805329					
	CC	673 (68.3)	1,264 (65.7)	Ref	
	CT	277 (28.1)	573 (29.8)	0.90	0.76-1.07
	TT	36 (3.7)	88 (4.6)	0.71	0.47-1.07
				P_{trend} = 0.07	

Abbreviations: OR, odds ratio; Ref, reference.
*Differences in numbers due to missing genotypes.
†Adjusted for age, gender, and study center.

wildtype (Table 3). This association was consistent by age of diagnosis (Supplementary Table 1), and the pattern was found for each of the four study centers (odds ratio$_{MDA}$, 1.18; odds ratio$_{NCI}$, 1.19; odds ratio$_{NIOSH}$, 1.38; odds ratio$_{UCSF}$, 1.78; Supplementary Table 1). The *G* allele frequency for controls of 35% is in the range reported for Caucasian populations (*G* allele range, 0.23-0.43) in the National Center for Biotechnology Information website.[10]

There were no significant associations overall between candidate single-nucleotide polymorphisms in the nucleotide excision repair pathway and glioblastoma multiforme. For the ERCC2 (rs13181) single-nucleotide polymorphism, we found a nonsignificant trend of increasing risk with the *C* allele (P_{trend} = 0.08). This trend reached statistical significance only when restricting to cases diagnosed at 50 years of age and older (P_{trend} = 0.03; Supplemental Table 1).

No significant differences for single gene associations were found by study center (Supplemental Table 1). Furthermore, no statistically significant two- or three-way gene interactions were found using the focused interaction testing framework when testing all genes or when restricting to genes in the same DNA repair pathways (data not shown).

Table 4 shows the haplotype-based analysis of the three DNA repair genes from chromosome 19 single-nucleotide polymorphisms (ERCC2 rs13181, ERCC1 rs3212986, and GLTSCR1 rs1035938) and glioblastoma multiforme. Although the three single-nucleotide polymorphisms were not in strong linkage disequilibrium, we assessed the joint effect of these single-nucleotide polymorphisms using a haplotype approach. A significant association was found between the most common haplotype (AGC) constructed from the chromosome 19 single-nucleotide polymorphisms (ERCC2 rs13181, ERCC1 rs3212986, and GLTSCR1 rs1035938) and risk for glioblastoma multiforme in comparison to all other haplotypes. Individuals with this haplotype were 23% less likely to be diagnosed with a glioblastoma multiforme than individuals with all other haplotypes combined (odds ratio, 0.77; 95% CI, 0.61-09.8; P = 0.03). There was no significant association between any of the individual three single-nucleotide polymorphisms and glioblastoma multiforme; however, each of the three variants was less frequent in the glioblastoma multiforme cases than the controls. There were no associations between any of the remaining haplotypes and glioblastoma multiforme in comparison to all other haplotypes combined or when individually compared with the most common haplotype.

Table 4. Haplotypes and risk for glioblastoma for loci at ERCC2, ERCC1, GLTSCR1, Glioblastoma Collaborative Group, 2008

	Haplotype*	Frequency	OR[†,‡]	95% CI	*P*
H1b	ACC	0.39	0.77	0.61-0.98	0.04
H2	ACT	0.152	1.19	0.85-1.68	0.32
H3	AAC	0.065	0.7	0.49-1.22	0.2
H4	AAT	0.027	1.04	0.44-2.70	0.93
H5	CCC	0.161	1.19	0.85-1.67	0.31
H6	CCT	0.052	1.28	0.69-2.37	0.44
H7	CAC	0.109	1.17	0.77-1.77	0.46
H8	CAT	0.044	1.34	0.66-2.71	0.42

*Haplotypes for loci at ERCC2 rs13181, ERCC1 rs3212986, and GLTSCR1 rs1035938.
†Odds ratio for haplotype compared with all other haplotypes.
‡Adjusted for age, gender, and center.

Discussion

Our results suggest that common variation in DNA repair genes may be associated with risk for glioblastoma multiforme. In the pooled analyses, we found two single-nucleotide polymorphisms (one in the base excision repair pathway and one in the nonhomologous end–joining pathway) associated with risk for glioblastoma multiforme. The *C* allele of the *PARP1* rs1136410 variant was associated with a 20% reduction in risk for glioblastoma multiforme. The *G* allele of the *PRKDC* rs7003908 variant was associated with a 44% increase in risk for glioblastoma multiforme, and there was a statistically significant trend (P = 0.009) with increasing number of *G* alleles. Although we found no individual associations between nucleotide excision repair candidate single-nucleotide polymorphisms and glioblastoma multiforme, we found a significant protective effect when three nucleotide excision repair single-nucleotide polymorphisms located near each other on chromosome 19 were modeled as a haplotype. The most common haplotype (AGC) when considering *ERCC2* rs13181, *ERCC1* rs3212986, and *GLTSCR1* rs1035938 was associated with a 23% reduction in risk (P = 0.03) compared with all other haplotypes combined. This finding may represent an additive effect of the individual single-nucleotide polymorphisms or the haplotype may be marking a region of chromosome 19 that contains another gene influencing risk for glioblastoma multiforme.

Although there are few published genetic association studies on gliomas, several articles have reported on associations between glioblastoma multiforme and the nucleotide excision repair pathway genes. Three analyses of chromosome 19 genes were published from the UCSF Genetic and Molecular Epidemiology of Adult Glioma Study. The *ERCC1* rs3212986 variant has been strongly associated with oligoastrocytomas but not with other types of gliomas. Using data collected through 1994, Chen et al. (29) found no association between the *ERCC1* rs3212986 *C* allele and risk for glioblastoma multiforme (odds ratio, 0.9; 95% CI, 0.5-1.6) but found a statistically significant association with oligoastrocytomas (odds ratio, 4.6; 95% CI, 1.6-13.2). The variant affects two proteins, ERCC1 and ASE-1, a nucleolar protein and T-cell receptor complex unit. From the same data set, Caggana et al. (30) found a significant association between a silent mutation (R156R) in *ERCC2* and all gliomas (glioblastoma multiforme, astrocytomas, oligoastrocytomas; odds ratio, 2.3; 95% CI, 1.3-4.2), whereas no significant association was found between the *ERCC2* rs13181 variant and glioblastoma multiforme (odds ratio$_{AC/CC\ versus\ AA}$, 1.6; 95% CI, 0.9-3.3). In our pooled analyses, there was a modest positive association between the *ERCC2* rs13181 *C* allele and risk for glioblastoma multiforme at each of the individual study

[10] http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?rs=7003908

centers (odds ratio$_{MDA}$, 1.18; odds ratio$_{NCI}$, 1.19; odds ratio$_{NIOSH}$, 1.13; odds ratio$_{UCSF}$, 1.25); however, the effect for the combined data did not reach statistical significance (odds ratio$_{AC/CC\ versus\ AA}$, 1.16; 95% CI, 0.99-1.37). An updated analysis of the UCSF data (using glioma cases identified through 1999) investigating *ERCC1* and *ERCC2* germline variants found no significant association between the *ERCC1* rs3212986 or the *ERCC2* rs13181 variants and risk for glioblastoma multiforme (18). A separate case-control study on chromosome 19 gene single-nucleotide polymorphisms and gliomas ($N = 141$) found that the *GLTSCR1* rs1035938 (*T* allele) and the *ERCC2* rs1052555 (*T* allele) single-nucleotide polymorphisms were significantly more common in oligodendroglioma cases than controls (31). Furthermore, the *GLTSCR1 T* allele was more common among cases with the 19q deletion than those without ($P = 0.01$).

Regions of chromosomal loss or gain of 19q have been described in studies on familial glioma (32, 33) and sporadic gliomas (34), suggesting that genes relevant to glioma risk are found in these regions. Investigators have observed differences in tissue expression and gene copy number for *ERCC1* and *ERCC2* in glioma (35, 36). These genes code for 2 essential proteins of 16 that contribute to DNA repair through the nucleotide excision repair pathway (37, 38). *ERCC2* is a 5′-3′ helicase that assists with DNA strand separation, and *ERCC1* is involved in the 5′ incision of DNA, which is necessary for the release of bulky DNA lesions (39, 40).

Two previous studies have described associations between polymorphisms in the *PRKDC* gene in the nonhomologous end–joining pathway with risk for gliomas. Wang et al. (41) investigated DNA repair genes that may be critical for the repair of DNA damage resulting from ionizing radiation, including *XRCC1, XRCC3, RAD51, p53*, and *PRKDC*. In this U.S. association study of 309 glioma patients and 342 cancer-free controls, investigators found a significant association between the *PRKDC* G6721T variant (rs7003908) and risk for glioma (41). The combined T variant (TT or GT) was associated with a 1.82-fold increase in risk for glioma (95% CI, 1.13-2.93; ref. 41). These findings contrast with the results of our current pooled analysis, wherein we found a 44% increase in risk associated with the *G* allele (odds ratio$_{GG\ versus\ TT}$, 1.44; 95% CI, 1.13-1.84). It is not clear why the two studies differ; however, our *T* allele frequency of 0.65 among controls and 0.63 among cases is consistent with the frequency described for Caucasians (T = 0.645) in the SNP500 National Center for Biotechnology Information website.[11] Liu et al. (42) completed a case-control study on 22 tagging single-nucleotide polymorphisms in nonhomologous end–joining pathway genes (*XRCC5, XRCC6*, and *PRKDC*) and gliomas; they found no association between any of the *PRKDC* tagging single-nucleotide polymorphisms and glioma risk. Specifically, there was no association between the *PRKDC* rs7003908 single-nucleotide polymorphism and risk for glioma in this Chinese population. In the analysis of single single-nucleotide polymorphisms, risk for glioma was associated with three tagging single-nucleotide polymorphisms (rs828704, rs3770502, rs9288516) in *XRCC5* and one tagging single-nucleotide polymorphism in *XRCC6* (rs6519265).

A recent publication of polymorphisms in DNA repair genes and gliomas found 16 single-nucleotide polymorphisms associated with glioma risk at the 1% significance level of 1,515 total single-nucleotide polymorphisms examined. The pooled analysis included data from 1,013 cases of all types of glioma and 1,016 controls combining data from five European population–based, case-control studies (43). The strongest association was found for the rs243356 single-nucleotide polymorphism in *CHAF1A* (odds ratio$_{trend}$, 1.32; 95% CI, 1.14-1.54; $P = 0.0002$). We did not examine variants in *CHAF1A* because the selection of single-nucleotide polymorphisms in our pilot study was completed before publication of the Bethke et al. (43) study. Of the 12 single-nucleotide polymorphisms included in our study on glioblastoma multiforme, the European study of glioma found a significant association with the *ERCC1* rs3212986 single-nucleotide polymorphism (odds ratio$_{trend}$, 0.81; 95% CI, 0.70-0.93; $P = 0.003$); no association was found for the *PARP1* rs1136410 ($P = 0.5$) or *PRKDC* rs7003908 single-nucleotide polymorphism ($P = 0.6$). Differences in findings between our glioblastoma multiforme pilot and the glioma study may reflect chance associations, differences in characteristics of the study populations, and/or the inclusion of different types of gliomas. Although it is possible that variation in DNA repair genes may be risk factors for all types of gliomas, it is also possible that some DNA repair single-nucleotide polymorphisms specifically contribute to glioblastoma multiforme.

Although we found a protective association with the *PARP1* rs1136410 *C* allele, other studies have found an increased risk for cancers of the prostate, esophagus, and lung with the *C* allele (44-46). The *PARP1* rs1136410 single-nucleotide polymorphism is a missense variant located in the catalytic domain of the gene (47, 48); the valine to alanine change is associated with a significant reduction in PARP1 enzymatic activity (49). Besides the catalytic domain at the C-terminal of the gene, other identifiable regions of PARP1 include an N-terminal DNA-binding domain, a nuclear localization sequence, and an internal automodification domain. Although the primary function of *PARP1* during DNA repair is the detection of DNA damage and the prevention of chromatid exchange, *PARP1* may also contribute to programmed cell death and to the up-regulation of an inflammatory response. The protective association found with the less active form of the enzyme could be explained by a diminished immune response among individuals with the *PARP1* variant. The absence of a dose-response in our findings may be chance or may reflect a dominant mode of action. *PARP1* is active in most cells found within the brain, including neurons, astrocytes, microglial cells, endothelia, and infiltrating leukocytes.

Of the 12 single-nucleotide polymorphisms we investigated in this initial pooled analyses, we found two single-nucleotide polymorphisms were associated with glioblastoma multiforme risk. These results may represent chance associations; however, the *PARP1* and *PRKDC* single-nucleotide polymorphisms remained significantly associated with risk after permutation testing. We constructed haplotypes for a set of three nucleotide excision repair single-nucleotide polymorphisms located

[11] http://snp500cancer.nci.nih.gov

near each other on chromosome 19. The individual single-nucleotide polymorphisms were not associated with risk, but the most common haplotype was associated with a 23% reduction in risk for glioblastoma multiforme compared with all other genotypes after adjusting for age, gender, and study center.

Because glioblastoma multiforme is a relatively rare cancer, it is difficult for any single study to recruit enough patients for genetic association and gene-gene interaction analyses. The pooling of data from four study centers allowed us to assemble a relatively large number of DNA samples for genetic association testing. The study represents one of the largest sets of glioblastoma multiforme cases assembled for genetic association testing to date. Although we did not conduct centralized pathology review for this study, the diagnosis of glioblastoma multiforme in the United States is considered consistent across time, geographic regions, and individual pathologists to allow for the use of existing diagnostic data for pooled analyses (50). We therefore felt confident that pooling of data from glioblastoma multiforme across study centers would result in a reasonably homogenous set of brain tumors for investigation. Collaboration of investigators across centers is critical for continued progress in brain tumor etiologic research. Another limitation of our study was the completion of genotyping at three laboratories and using more than one genotyping platform; to improve the interpretability of the combined results, quality control measures were planned in the design, including the incorporation of DNA standards at each study center with known genotypes.

In summary, our single single-nucleotide polymorphism and haplotype findings suggest that DNA repair variants in the nucleotide excision repair, base excision repair, and nonhomologous end–joining pathways may play an important role in the etiology of glioblastoma multiforme. Additional studies investigating more comprehensive sets of genes and their impact on survival, as well as etiology, are needed. This study is one of the largest association studies on adult glioblastoma multiforme, the most common and deadly form of adult brain cancer. Collaborative studies investigating genetic and environmental risk and prognostic factors are needed to extend the length and quality of life for patients with glioblastoma multiforme and other types of gliomas.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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