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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 (GBM), the most common and deadliest form of adult brain tumors. Genetic data for approximately 1,000 GBM cases and 2,000 controls were combined from four centers in the United States that have conducted case-control studies of adult GBM 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 SNPs 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 of GBM ($OR_{CT\ or\ CC}$ =0.80; 95%CI 0.67–0.95). A 44% increase in risk of GBM was found for individuals homozygous for the G allele of the *PRKDC* rs7003908 variant (OR_{GG} 1.44; 95%CI 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 3 SNPs (*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 to 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 GBMs. Our results suggest that common variation in DNA repair genes may be associated with risk of GBMs.

Keywords

DNA repair; glioblastoma; brain tumor; polymorphisms; pooled

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Introduction

Glioblastoma multiforme (GBM) 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 GBMs are largely unknown with no single risk factor identified that explains a large proportion of cases. While 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's 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 GBM therefore, remains of critical importance.

Deficiencies in DNA repair pathways have been associated with cancer risk, with 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 due to 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, such as pesticides, *N*-nitroso compounds (NOCs), 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 (BER) is a multi-step process for the removal of small base adducts such as those produced by methylation or oxidation (10). Nucleotide excision repair (NER) corrects UV-induced lesions, intra-strand 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 NOCs (11–13). Direct repair is another mechanisms that acts to reverse rather than excise DNA damage, typically involving methyl and other small alkyl groups (8). More severe double strand breaks (DSBs) may occur following exposure to ionizing radiation or to products of cellular processes resulting in hydrolysis, oxidation, or methylation of DNA. Non-homologous end-joining (NHEJ) is believed to be the primary repair pathway for the majority of DSBs, including those resulting from ionizing radiation (14), Homologous recombination (HR) is an alternative repair mechanism for DSBs; 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 GBMs. In the current analysis, we examined the association between select variants in DNA repair genes and risk of GBM. We combined genetic data using DNA specimens from four centers in the United States that have conducted studies of gliomas 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. We selected 12 putative functional SNPs 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 approximately 1,000 GBM cases and 2,000 controls.

Material and Methods

Study Population

Data for these analyses were assembled from four existing case-control studies of brain tumors in the United States: (1) The Multicenter Study of Environment and Health, by investigators at the National Cancer Institute (NCI) (2) The Upper Midwest Health Study, by investigators at the National Institute for Occupational Safety and Health (NIOSH) of the Centers for Disease Control and Prevention (CDC) (3) The Family Health Study, by investigators at The University of Texas M.D. Anderson Cancer Center, and (4) The Genetic and Molecular Epidemiology of Adult Glioma Study, by investigators at the University of California at San Francisco (UCSF). The collaborative effort was established through meetings organized by the Brain Tumor Epidemiology Consortium (BTEC), an international organization established in 2003 to develop multi-center and interdisciplinary collaborations that will lead to a better understanding of the etiology, outcomes, and prevention of brain tumors[1].

Twelve SNPs in 11 candidate DNA repair genes were selected for testing in the pilot study (see 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, NOCs) or products of cellular metabolic processes. We selected SNPs 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 of glioma in the literature. All SNPs had minor allele frequencies >0.1. Five SNPs were selected from the BER pathway (OGG1 rs1052133; APEX1 rs1130409; XRCC1 rs25487; XRCC1 rs1799782; PARP rs1136410), 5 SNPs were selected from the NER pathway (ERCC2 rs13181; RAD23B rs1805329; ERCC5 rs17655; GLTSCR1 rs1035938; ERCC1 rs3212986), 1 SNP was selected from the direct repair pathway (MGMT rs12917) and 1 SNP from the NHEJ pathway (PRKDC rs7003908).

Existing DNA samples and demographic data for 1,015 cases of GBM and 1,994 controls from the 4 study centers were identified for inclusion in the pooled analyses. Cases for the combined analyses include adults, 18 years of age and older at the time of diagnosis with histologically confirmed primary glioblastoma (ICD-O 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 and were identified from 3 hospitals in Phoenix, Arizona; Boston, Massachusetts; and Pittsburgh, Pennsylvania from 1994–1998. 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, non-metropolitan counties in Iowa, Michigan, Minnesota, and Wisconsin who were diagnosed with a glioma from 1995–1997. 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, an identity card, or to be enrolled in Medicare to maintain comparability to controls (20).
3. The Harris County Case-Control Study (UT MD Anderson Cancer Center): cases were identified through physician or clinic referrals at hospitals where brain tumor

[1]Brain Tumor Epidemiology Consortium (BTEC). http://epi.grants.cancer.gov/btec/

patients are diagnosed in Harris 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 GBM residing in 6 San Francisco Bay Area counties (San Francisco, Alameda, Contra Costa, Santa Clara, San Mateo, Marin) were ascertained between 1991–1994, 1997–1999 and 2001–2004 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 University of California, San Francisco Neuro-oncology clinic from 2002–2006 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 5 aliquots of blood were eligible for this present study in order to conduct the additional genotyping; thus, the numbers of participants are lower than the totals from other papers published from this group.

In person interviews were used to collect demographic, lifestyle, and environmental exposure histories at each of the 4 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, MD Anderson, 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 white blood cells using a phenol-chloroform method (23) and genotyped for the DNA repair single nucleotide polymorphisms (SNPs) using custom designed TaqMan genotyping assays (Applied Biosystems, Foster City, CA) at the NCI Core Genotyping Facility (Gaithersburg, MD). At UCSF, DNA was extracted from heparinized whole blood using Qiagen column purification (Valencia, CA) or Autogen DNA (AutoGen, Holliston, MA). Genotyping for the majority of samples was completed using the same custom designed TaqMan assays (Applied Biosystems, Foster City, CA) as 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 SNP assay panel (24–26). At MD Anderson, genoraic DNA was extracted from peripheral blood lymphocytes using the Qiagen Blood Kit (Valencia, CA). Genotyping was performed using the Sequenom MassARRAY iPLEX™ platform[2]. 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 Biorespository) were included in the genotyping assays at the 3 laboratories. At the NCI laboratory, an additional 85 DNA standard controls (100 total) (Coriell Biorepository) were included in the pilot genotyping plates.

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

Statistical analyses

The distribution of allele frequencies for cases and controls was examined overall and by study center. Pearson chi-square tests were used to assess the consistency of allele frequencies with Hardy-Weinberg equilibrium among the controls. Summary and stratum-specific odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using unconditional logistic regression to evaluate the association between each DNA repair SNP and risk of glioblastoma for all samples and by age (<50, 50+ years 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 GBM risk factors including education and family history of cancer or brain cancer was completed however these variables were not included in the final models as 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 SNP association results, we conducted permutation testing. In brief, 10000 datasets were generated through Monte Carlo sampling. Each of these datasets was analyzed in a manner identical to that of the original, un-permuted 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 (FITF) (27). Interactions were evaluated considering all 12 SNPs and after restricting to SNPs in common pathways (e.g. BER, NER), Likelihood-ratio tests were performed in stages that increase in the order of interaction considered. Joint tests of main effects and interactions were performed conditional on significant lower-order effects. A reduction in the number of tests performed is achieved by prescreening gene combinations with a goodness-of-fit chi-square statistic 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 performed using 3 NER pathway SNPs 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 snps were not included as they are in the BER pathway and not in close proximity of the NER 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 individual's genotype. The estimates were used within logistic regression models to estimate ORs and 95% CIs.

Calculations were performed using PROC LOGISTIC (SAS V 9.1) and PROC HAPLOTYPE from SAS/Genetics (SAS Institute, Cary, NC, USA).

Results

A total of 1,015 GBM cases and 1,994 controls were genotyped for 12 SNPs in the DNA repair candidate pathways. Of these, 213 were contributed by MD Anderson, 171 from NCI, 139 from NIOSH, and 492 from UCSF (see Table 2). In the pooled data, the majority of 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% vs. 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).

All SNPs were consistent with Hardy Weinberg equilibrium (HWE) for controls at each study center. Data from the 4 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 SNPs. 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 individuals SNPs ranged from 96% (GLTSCR, ERCC1) to 99% (ERCC2). Numbers of individuals genotyped for each SNP are shown in Table 3.

Among the 5 BER genes, only the PARP1 SNP (rs1136410) was significantly associated with risk of GBM. No significant associations were found for the remaining 4 BER SNPs (APEX1 rs1130409, OGG1 rs1052133, XRCC1 rs25487, XRCC1 rs1799782) or for the single direct repair SNP (MGMT rs 12917) (Table 3). Participants with one or two copies of the PARP1 C allele were at lower risk of a GBM than participants homozygous for the T aliele ($OR_{CT\ or\ CC}$= 0.80; 95%CI 0.67–0.95). Risk was similar for individuals heterozygote or homozygous for the C allele (OR_{CT}= 0.79; 95%CI 0.67–0.95 and OR_{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 NCBI website for Caucasian populations.[3] We found a significant trend in risk by genotype that was present for individuals 50 years of age and older but not for individuals less than 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 GBM than individuals with the wildtype (Table 3). This association was consistent by age of diagnosis (Supplementary Table 1) and the pattern was found for each of the 4 study centers (OR_{MDA}=1.18, OR_{NCI}=1.19, OR_{NIOSH}=1.38, OR_{UCSF}=1.78) (Supplementary Table 1). The G allele frequency for controls of 35% is in the range reported for Caucasian populations (G allele ranges from .23–0.43) in the NCBI website[4].

There were no significant associations overall between candidate SNPs in the NER pathway and GBM. For the ERCC2 (rs13181) SNP, we found a non-significant 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 2 or 3-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 3 DNA repair genes from chromosome 19 SNPs (ERCC2 rs13181; ERCC1 rs3212986, and GLTSCR1 rs1035938) and GBM. Although the 3 SNPs were not in strong linkage disequilibrium, we assessed the joint effect of these SNPs using a haplotype approach. A significant association was found between the most common haplotype (AGC) constructed from the chromosome 19 SNPs (ERCC2 rs 13181; ERCC1 rs3212986, and GLTSCR1 rs1035938) and risk of GBM in comparison to all other haplotypes. Individuals with this haplotype were 23% less likely to be diagnosed with a GBM than individuals with all other haplotypes combined (OR=0.77; 95%CI 0.61, 09.8, p=0.03). There was no significant association between any of the individual 3 SNPs and GBM; however,

[3]http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?type=rs&rs=1136410
[4]http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?rs=7003908

each of the 3 variants was less frequent in the GBM cases than the controls. There were no associations between any of the remaining haplotypes and GBM in comparison to all other haplotypes combined or when individually compared to the most common haplotype.

Discussion

Our results suggest that common variation in DNA repair genes may be associated with risk of GBMs. In the pooled analyses, we found two SNPs (one in the BER pathway and one in the NHEJ pathway) associated with risk of GBM. The C allele of the *PARP1* rs1136410 variant was associated with a 20% reduction in risk of GBM. The G allele of the *PRKDC* rs7003908 variant was associated with a 44% increase in risk of GBM, and there was a statistically significant trend (p=0.009) with increasing number of G alleles. While we found no individual associations between NER candidate SNPs and GBM, we found a significant, protective effect when 3 NER SNPs located near each other on chromosome 19 were modeled as a haplotype. The most common haplotype (AGC) when considering *ERCC2* rs13l81, *ERCC1* rs3212986, and *GLTSCR1* rs1035938 was associated with a 23% reduction in risk (p=0.03) compared to all other haplotypes combined. This finding may represent an additive effect of the individual SNPs or the haplotype may be marking a region of chromosome 19 that contains another gene influencing risk of GBM.

While there are few published genetic association studies of gliomas, several articles have reported on associations between GBM and the NER 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. (2000) found no association between the *ERCC1* rs3212986 C allele and risk of GBMs (OR=0.9; 95%CI 0.5, 1.6), but found a statistically significant association with oligoastrocytomas (OR=4.6; 95%CI 1.6, 13.2) (29). The variant affects two proteins, ERCC1, and ASE-1, a nucleolar protein and T-cell receptor complex unit. From the same dataset, Cagganna et al. (2001) found a significant association between a silent mutation (R156R) in *ERCC2* and all gliomas (GBM, astrocytomas, oligoastrocytomas) (OR=2.3; 95%CI 1.3, 4.2), while no significant association was found between the *ERCC2* rs13181 variant and GBM ($OR_{AC/CC}$ vs. $_{AA}$=l.6; 95%CI 0.9, 3.3) (30). In our pooled analyses, there was a modest, positive association between the *ERCC2* rs13181 C allele and risk of GBM at each of the individual study centers (OR_{MDA}=1.18, OR_{NCI}=1.19, OR_{NIOSH}=1.13, OR_{UCSF}=l.25); however, the effect for the combined data did not reach statistical significance ($OR_{AC/CC}$ vs. $_{AA}$=1.16; 95% CI 0.99, 1.37). An updated analysis of the UCSF data (using glioma cases identified through 1999) investigating both *ERCC1* and *ERCC2* germline variants found no significant association between the *ERCC1* rs3212986 or the *ERCC2* rs13181 variants and risk of GBM (18). A separate case-control study of chromosome 19 gene SNPs and gliomas (N=141) found the *GLTSCR1* rs1035938 (T allele) and the *ERCC2* rs 1052555 (T allele) SNPs were significantly more common in oligodendroglioma cases than controls (31). Further, 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 of 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 out of 16 that contribute to DNA repair through the NER 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 NHEJ pathway with risk of gliomas. Wang et al. (2004) 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* (41). 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 of glioma (41). The combined T variant (TT or GT) was associated with a 1.82-fold increase in risk of glioma (95% CI 1.13, 2:93) (41). These findings contrast with the results of our current pooled analysis where we found a 44% increase in risk associated with the G allele (OR_{GG} vs. $_{TT}$=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 NCBI website[5]. Liu et al. (2007) completed a case-control study of 22 tagging SNPs (tSNPs) in NHEJ pathway genes (*XRCC5, XRCC6, and PRKDC*) and gliomas; they found no association between any of the *PRKDC* tSNPs and glioma risk. Specifically, there was no association between the *PRKDC* rs7003908 SNP and risk of glioma in this Chinese population. In the analysis of single SNPs, risk of glioma was associated with 3 tSNPs (rs828704, rs3770502, rs9288516) in *XRCC5* and one tSNP in *XRCC6* (rs6519265) (42).

A recent publication of polymorphisms in DNA repair genes and gliomas found 16 SNPs associated with glioma risk at the 1% significance level out of 1,515 total SNPs examined. The pooled analysis included data from 1,013 cases of all types of glioma and 1,016 controls combining data from 5 European population-based, case-control studies (43). The strongest association was found for the rs243356 SNP in *CHAF1A* (OR_{trend}=1.32; 95%CI 1.14, 1.54; p=0.0002). We did not examine variants in *CHAF1A* as the selection of SNPs in our pilot study was completed before publication of the Bethke et al. (2008) study. Of the 12 SNPs included in our study of GBM, the European study of glioma found a significant association with the *ERCC1* rs3212986 SNP (OR_{trend}=0.81; 95%CI 0.70–0.93; p=0.003); no association was found for the *PARP1* rs 1136410 (p=0.5) or *PRKDC* rs7003908 SNP (p=0.6). Differences in findings between our GBM 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. While 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 SNPs specifically contribute to GBM.

While we found a protective association with the *PARP1* rs1136410 C allele, other studies have found an increased risk of cancers of the prostate, esophagus, and lung with the C allele (44–46). The *PARP1* rs1136410 SNP 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 auto-modification domain. While 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 upregulation 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 SNPs we investigated in this initial pooled analyses, we found 2 SNPs were associated with GBM risk. These results may represent chance associations; however, both the *PARP1* and *PRKDC* SNPs remained significantly associated with risk after permutation testing. We

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

constructed haplotypes for a set of 3 NER SNPs located near each other on chromosome 19. The individual SNPs were not associated with risk, but the most common haplotype was associated with a 23% reduction in risk of GBM compared to all other genotypes after adjusting for age, gender, and study center.

Because GBM 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 GBM cases assembled for genetic association testing to date. Although we did not conduct centralized pathology review for this study, the diagnosis of GBM in the United States is considered consistent across time, geographical 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 GBMs 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 3 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 SNP and haplotype findings suggest that DNA repair variants in the NER, BER, and NHEJ pathways may play an important role in the etiology of GBM. 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 of adult GBMs, 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 GBMs and other types of gliomas.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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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

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[a] (mean±SD)	56.3 ± 12.6		53.6 ±15.3	

[a] age at diagnosis for cases and reference age for controls

Table 3

Odds Ratios and 95%CI for DNA repair SNPs and risk of glioblastoma for all centers combined, Glioblastoma Collaborative Group, 2008

Gene SNP	genotype	CA[b] (%)	CO[b] (%)	OR[c]	95%CI
Base Excision Repair					
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)	1150 (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)	1303 (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)	1664 (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)	1480 (75.2)	ref	

Gene SNP	genotype	CA[b] (%)	CO[b] (%)	OR[c]	95%CI
	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
Non Homologous End Joining					
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
Nucleotide Excision Repair					
ERCC1 *rs3212986*					
	CC	557 (57.0)	1087 (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)	1017 (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*					

Gene SNP	genotype	CA[b] (%)	CO[b] (%)	OR[c]	95%CI
	CC	673 (68.3)	1264 (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

[b] differences in numbers due to missing genotypes;

[c] adjusted for age, gender and study center.

Table 4

Haplotypes and risk of glioblastoma for loci at ERCC2, ERCC1, GLTSCR1, Glioblastoma Collaborative Group, 2008

	Haplotype[a]	Frequency	OR[b,c]	95%CI	p-value
H1[b]	**A-C-C**	**0.39**	0.77	(0.61,0.98)	0.04
H2	A-C-T	0.152	1.19	(0.85, 1.68)	0.32
H3	A-A-C	0.065	0.7	(0.49, 1.22)	0.2
H4	A-A-T	0.027	1.04	(0.44, 2.70)	0.93
H5	C-C-C	0.161	1.19	(0.85, 1.67)	0.31
H6	C-C-T	0.052	1.28	(0.69, 2.37)	0.44
H7	C-A-C	0.109	1.17	(0.77, 1.77)	0.46
H8	C-A-T	0.044	1.34	(0.66, 2.71)	0.42

[a] Haplotypes for loci at ERCC2 rs13181, ERCC1 rs3212986, GLTSCR1 rs1035938

[b] Odds ratio for haplotype compared to all other haplotypes.

[c] Adjusted for age, gender, and center.