Association and Interactions between DNA Repair Gene Polymorphisms and Adult Glioma

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Abstract

It is generally accepted that glioma develops through accumulation of genetic alterations. We hypothesized that polymorphisms of candidate genes involved in the DNA repair pathways may contribute to susceptibility to glioma. To address this possibility, we conducted a study on 373 Caucasian glioma cases and 365 cancer-free Caucasian controls to assess associations between glioma risk and 18 functional single-nucleotide polymorphisms in DNA repair genes. We evaluated potential gene-gene and gene-environment interactions using a multianalytic strategy combining logistic regression, multifactor dimensionality reduction and classification and regression tree approaches. In the single-locus analysis, six single-nucleotide polymorphisms [*ERCC1* 3′ untranslated region (UTR), *XRCC1* R399Q, *APEX1* E148D, *PARP1* A762V, *MGMT* F84L, and *LIG1* 5′UTR] showed a significant association with glioma risk. In the analysis of cumulative genetic risk of multiple single-nucleotide polymorphisms, a significant gene-dosage effect was found for increased glioma risk with increasing numbers of adverse genotypes involving the aforementioned six single-nucleotide polymorphisms (P_{trend} = 0.0004). Furthermore, the multifactor dimensionality reduction and classification and regression tree analyses identified *MGMT* F84L as the predominant risk factor for glioma and revealed strong interactions among ionizing radiation exposure, *PARP1* A762V, *MGMT* F84L, and *APEX1* E148D. Interestingly, the risk for glioma was dramatically increased in ionizing radiation exposure individuals who had the wild-type genotypes of *MGMT* F84L and *PARP1* A762V (adjusted odds ratios, 5.95; 95% confidence intervals, 2.21-16.65). Taken together, these results suggest that polymorphisms in DNA repair genes may act individually or together to contribute to glioma risk. (Cancer Epidemiol Biomarkers Prev 2009;18(1):204–14)

Introduction

Malignant gliomas are the most common primary brain tumor in adults. Few factors have thus far been conclusively shown to affect glioma risk, namely, family history, rare genetic syndromes, and exposure to high doses of ionizing radiation. However, these factors only account for a small proportion of cases (1-4). Therefore, the most generally accepted model of carcinogenesis postulates that glioma develops through accumulation of genetic alterations that allow the cells to escape normal growth-regulatory mechanisms (5).

It is increasingly clear that genetic susceptibility to cancer is complex because of interactions between and among genes and environmental factors. Such interactions are ubiquitous to complex genetic diseases such as cancer (6), and association studies are designed to address this complexity. Molecular epidemiologic studies have moved from the evaluation of a single candidate gene to the consideration of a pathway comprising dozens of genes and their environmental factors, even to multiple pathways that form complex networks.

DNA is continually subjected to a variety of assaults, as a result of internal, cellular metabolic processes and exposure to genotoxic or clastogenic agents. Efficient and proficient DNA repair is thus required for the effective maintenance of genome integrity. Such DNA damage requires the concerted actions of a number of DNA repair genes to restore genomic integrity, including the nucleotide excision repair, base excision repair, Double Strand Break (DSB) repair, and mismatch repair pathways, as well as direct reversal of damage. Thus, common polymorphisms of DNA repair genes are plausible candidates that may contribute to susceptibility to glioma. Whereas several studies have investigated the role of single-nucleotide polymorphisms in DNA repair genes and susceptibility to glioma and the results are encouraging (7-11), few have systematically examined glioma risk in the context of single-nucleotide polymorphisms in the different DNA repair pathways together (12, 13). Hence, we tested the hypothesis that polymorphisms of candidate genes involved in the DNA repair pathway genes may contribute to susceptibility to glioma. In this study, we used a candidate pathway-based approach to investigate 18 potential functional polymorphisms in 12 key genes in the different DNA repair pathways, including nucleotide excision repair

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pathway, base excision repair pathway, DSB repair pathway, and direct reversal of damage. Furthermore, based on the interaction of these genes at the molecular level and the possibility that these interactions might be modified by specific environmental factors, we also tested the hypothesis that gene-gene, gene-environment interactions contribute to glioma susceptibility using a multianalytic strategy that combined traditional statistical methods with novel computational algorithms.

Materials and Methods

Study Population. Cases were Caucasian adults (18 y or older) with newly diagnosed gliomas (International Classification of Diseases 9 codes 9380-9481) residing in 15 counties surrounding Harris County, Texas (Austin, Brazoria, Chambers, Colorado, Fort Bend, Galveston, Harris, Jefferson, Liberty, Montgomery, Orange, San Jacinto, Walker, Waller, and Wharton), and recruited into the study between January 2001 and January 2006. A pathology specimen was obtained for all patients, and glioma diagnosis was confirmed by a neuropathologist. Glioma cases with previous diagnosis of cancers were excluded. Patients signed an informed consent and were interviewed to obtain the information described below. Proxies were interviewed if the patients were unable to participate because of cognitive disability. We exclude minority patients for the analysis because only a small number of glioma patients were reached (15 African-American and 34 Hispanic). Controls were frequency matched to cases on age (±5 y), gender, and race or ethnicity. Controls were all English-speaking residents of the same Houston area counties and were recruited through random-digit dialing through a contracting company using standard methods (14, 15). Controls must have not been previously diagnosed with cancer at the time of enrollment. Eligible controls were contacted to obtain informed consent and schedule an interview. The participation rates for cases and controls were 77% and 83%, respectively.

Cases and controls underwent a detailed interview, either in person or over the telephone. The following information were collected: demographic variables, cigarette smoking and alcohol consumption, family history of brain tumor (first degree) or other cancers, and ionizing radiation exposure histories (medical and occupational). The reported exposures to ionizing radiation are likely to cover a wide range of doses. Medical ionizing radiation exposure history was assessed by collecting information on diagnostic (that is, routine chest X-rays) and any radiotherapy for a medical problem (such as acne or hyperthyroidism). For the diagnostic exposure, details such as the dates, site of body, and reasons for exposure were obtained. For therapeutic exposure, details such as the nature of the disease requiring radiation, the number of treatment cycles, area of the body that received irradiation, and age at initiation and at the end of the treatments. Only exposures that had occurred at least 2 y before diagnosis (cases) or reference date (interview for the controls) were included as ''exposed.'' Occupational ionizing radiation exposure included work as a pilot, flight attendant, astronaut, uranium miner, workers in the nuclear power industries, radiologists or X-ray medical worker, dentist or dental hygienist, and other participants who self-reported ionizing radiation exposure in the workplace. We only included occupational exposures of at least 1 y in duration for a minimum of 2 y before the reference date (diagnosis for cases; interview for controls). A 20-mL blood specimen was obtained from all patients and control subjects. The research protocol was approved by the University of Texas M. D. Anderson institutional review board.

Selection of Candidate Single-Nucleotide Polymorphisms and Genotyping. Candidate single-nucleotide polymorphisms were selected from the best evidence from published studies, to represent more commonly occurring variants (minor allele frequencies, >10%) and to gain statistical power to detect interactions. We selected a total of 18 literature-defined putative functional polymorphisms in 12 key genes (16-20), 6 single-nucleotide polymorphisms in the nucleotide excision repair pathway (*XPC* V499A rs2228000, *XPC* Q939K rs2228001, *XPD/ERCC2* R156R rs238406, *XPD* Q751K rs13181, *XPG/ERCC5* H1104D rs17655, *ERCC1* 3′UTR rs3212986), 5 single-nucleotide polymorphisms in the base excision repair pathway (*XRCC1* W194R rs1799782, *XRCC1* R399Q rs25487, *OGG1* C326S rs1052133, *PARP*1 A762V rs1136410, *APEX*1 E148D rs3136819), 3 single-nucleotide polymorphisms in direct reversal of damage pathway (*MGMT* F84L rs12917, V143I rs2308321, and R178K rs2308327), 2 single-nucleotide polymorphisms in DSB repair pathway (*XRCC3* T241M rs861539 and *NBS* Q185E rs1805794), and 2 single-nucleotide polymorphisms in other repair genes (*LIG*1 5′UTR rs20579 and *LIG*1 A170A rs20580).

Genomic DNA was extracted from peripheral blood lymphocytes using the Qiagen Blood Kit (Qiagen, Inc.). Genotyping was done using the Sequenom MassARRAY iPLEX platform according to the manufacturer's instructions (http://www.sequenom.com/sEq.genotyping.html). The quality control analysis included the genotyping of internal positive control samples, the use of no template controls, and the use of replicates for 10% of the samples.

Statistical Analysis. Goodness of fit to the Hardy-Weinberg equilibrium expectation in control subjects was assessed by the χ^2 test for each single-nucleotide polymorphism. Genotype frequencies in cases and control subjects were compared using the χ^2 test. Odds ratios and 95% confidence intervals (95% CI) were calculated by unconditional logistic regression analysis with adjustment for age and gender. The Akaike's information criterion was used to determine the best genetic model for each single-nucleotide polymorphism (21). Besides the permutation testing (1,000 times), we also adopted a powerful bootstrapping method to reduce the potential for spurious findings due to multiple testing and to validate the results in our sample (22). The bootstrap approach selects random samples of size N (cases + controls) with replacement from the original data (23, 24). In our study, within each bootstrap replicate (1,000 replications), we chose a random sample (each random sample comprised 373 cases and 365 controls), which allows for any one participant to be chosen once, more than once, or not at all for each replicate. We then calculated the odds ratio for each

replicate and constructed an empirical distribution for the odds ratio. We calculated the mean odds ratio across all replicates and obtained 2.5% and 97.5% percentiles of the empirical distribution to construct a 95% CI for the odds ratio. All statistical tests were two sided.

We examined linkage disequilibrium among the polymorphisms using Lewontin's standardized coefficient D' and the linkage disequilibrium coefficient r^2 (25). We used the HAPLO.STATS package developed by Schaid et al. (http://www.mayo.edu/hsr/Sfunc.html; ref. 26) for the haplotype analysis. This method, which is based on the generalized linear model framework, allows for the adjustment of possible confounding variables and provides both global and haplotype-specific tests. Haplotypes with a frequency of <0.03 were pooled into a combined group. Empirical *P*s, based on 1,000 simulations, were computed for the global score test and each of the haplotype-specific score tests.

We used a multiphase strategy for analyzing gene-gene and gene-environment interactions. First, using multivariate logistic regression, global effect interactions by the number of adverse genotypes identified from the single locus analysis were examined. Then, we applied the multifactor dimensionality reduction method to identify interaction models. The nonparametric and genetic model–free multifactor dimensionality reduction analysis was done using version 0.5.1 of the open-source multifactor dimensionality reduction software package that is available online.[6] The multifactor dimensionality reduction approach is described in detail by Ritchie et al. (27) and reviewed by Moore et al. (6, 28). In this study, the multifactor dimensionality reduction analysis was conducted using the dichotomous groupings of the polymorphisms selected by the Akaike's information criterion, and we used 100-fold cross-validation consistency and 1,000-fold permutation testing. Multifactor dimensionality reduction results were considered statistically significant at the 0.05 level. To better confirm and visualize the interaction models identified by multifactor dimensionality reduction, we further built an entropy-based interaction dendrogram (29, 30). This would enable the attributes (that is, single-nucleotide polymorphisms) that strongly interact to appear close together at the leaves of the tree with those not interacting, appearing distant from one another. Lastly, we conducted a classification and regression tree analysis to detect and characterize the high-order interactions using the Helix-Tree Genetics Analysis software (version 4.1.0; Golden Helix). Classification and regression tree is a binary recursive partitioning method that produces a decision tree to identify subgroups of subjects at higher risk (31). Specifically, the recursive partitioning algorithm in Helix-Tree starts at the root node (with the entire data set) and uses a statistical hypothesis testing method (that is, formal inference-based recursive modeling) to determine the first locally optimal split and each subsequent split of the data set, with multiplicity-adjusted *P*s to control tree growth ($P < 0.05$). This process continues until the terminal nodes have no subsequent statistically significant splits or the terminal nodes reach a pre-specified minimum size (at least 10 subjects for each terminal node). Subgroups of individuals with differential risk associations were identified in the different nodes of the tree, indicating the presence of interactions. In our analyses, the single-nucleotide polymorphism variables were considered as two categories according to their Akaike's information criterion selected best-effect model. Reference group is the least percentage of cases. Odds ratio and 95% CI were adjusted by age and gender.

[6] http://www.epistasis.org/software.html

Results

Characteristics of Study Subjects. Our analysis included 373 Caucasian glioma cases, including 33 (4%) proxy-reports, and 365 cancer-free Caucasian controls. The characteristics of cases and control subjects are summarized in Table 1. The case group had more males than the control group (56.8% versus 43.6%) and were more likely than the controls to report a family history of brain tumor (4.0% versus 2.7%) in their first-degree relatives. With regard to ionizing radiation exposure, 35 glioma cases (8 medical exposures, 27 occupationally exposed) and 21 cancer-free controls (2 medical exposures, 19 occupationally exposed) reported a history of ionizing radiation exposure (9.4% versus 5.9%). For participants undergoing medical radiation treatment, the diagnoses were severe acne (2 cases and 1 control), hyperthyroid (2 cases), birth mark (1 case and 1 control), thick sinus mucus (1 case), Bell palsy (1 case), and tonsillitis (1 case). More than two thirds of the 46 occupationally exposed participants were in the medical field (physicians, radiologists, and nurses). Among the exposed professions were also pilots and engineers. Of the 373 cases, 214 were glioblastoma, categorized as high-grade glioma; 77 were anaplastic astrocytoma, medium-grade glioma; and 82 were other low-grade

Table 1. Frequency distribution of selected characteristics of study subjects by the case-control status

Variable	Cases (n = 373)	Controls (n = 365)
	No. (%)*	No. (%)*
Age (y)		
≤45	142 (38.1)	143 (39.2)
>45	231 (61.9)	222 (60.8)
Gender		
Male	212 (56.8)	159 (43.6)
Female	161 (43.2)	206 (56.4)
Smoking status		
Never	222 (59.7)	205 (56.2)
Ever	150 (40.3)	160 (43.8)
Family history of brain tumor		
No	339 (96.0)	326 (97.3)
Yes	14 (4.0)	9 (2.7)
IR exposure history		
No	337 (90.6)	337 (94.1)
Yes	35 (9.4)	21 (5.9)
Histology type †		
High grade	214 (57.4)	
Medium grade	77 (20.6)	
Low grade	82 (22.0)	

Abbreviation: IR, ionizing radiation.
*Numbers do not add up to the column totals because of missing values.
† High-grade glioma (glioblastoma); medium-grade glioma (anaplastic astrocytoma); low-grade glioma (oligodendroglioma, not-otherwise-specified astrocytoma, and mixed glioma).

Table 2. Genotype frequencies of 18 single-nucleotide polymorphisms among cases and controls and their associations with risk for glioma

Pathway	Gene and SNP	Genotype	No. (frequency)		Logistic regression OR (95% CI)*	Bootstrap OR (95% CI)†
			Cases	Controls		
NER						
DM	*XPC* V499A	CC	220 (59.9%)	206 (56.6%)	1.00 (reference)	1.00 (reference)
	rs2228000	CT/TT	147 (40.1%)	158 (43.4%)	0.85 (0.63-1.16)	0.87 (0.63-1.18)
	XPD R156R	GG	123 (33.0%)	101 (27.7%)	1.00 (reference)	1.00 (reference)
	rs238406	GT/TT	250 (67.0%)	263 (72.3%)	0.77 (0.56-1.05)	0.78 (0.56-1.05)
	XPD Q751K	TT	139 (37.9%)	161 (44.5%)	1.00 (reference)	1.00 (reference)
	rs13181	TG/GG	228 (62.1%)	201 (55.5%)	1.30 (0.96-1.75)	1.31 (0.97-1.74)
RM	*XPC* Q939K	AA/AC	315 (84.9%)	297 (81.4%)	1.00 (reference)	1.00 (reference)
	rs2228001	CC	56 (15.1%)	68 (18.6%)	0.75 (0.50-1.11)	0.76 (0.49-1.10)
	XPG H1104D	CC/CG	353 (94.6%)	351 (96.4%)	1.00 (reference)	1.00 (reference)
	rs17655	GG	20 (5.4%)	13 (3.6%)	1.47 (0.71-3.05)	1.61 (0.69-3.36)
	ERCC1 3′UTR	GG/GT	338 (91.6%)	345 (95.3%)	1.00 (reference)	1.00 (reference)
	rs3212986	TT	31 (8.4%)	17 (4.7%)	1.86 (1.01-3.46)	1.97 (1.01-3.73)
BER						
DM	*XRCC1* R399Q	GG	149 (39.9%)	169 (46.4%)	1.00 (reference)	1.00 (reference)
	rs25487	GA/AA	224 (60.1%)	195 (53.6%)	1.43 (1.05-1.92)	1.44 (1.06-1.92)
	PARP1 A762V	TT	267 (71.8%)	236 (64.7%)	1.00 (reference)	1.00 (reference)
	rs1136410	TC/CC	105 (28.2%)	129 (35.3%)	0.71 (0.52-0.97)	0.72 (0.52-0.99)
RM	*XRCC1* W194R	CC/CT	209 (99.7%)	362 (99.2%)	1.00 (reference)	1.00 (reference)
	rs1799782	TT	1 (0.3%)	3 (0.8%)	0.52 (0.07-5.26)	N.A
	OGG1 C326S	CC/CG	355 (95.4%)	345 (94.5%)	1.00 (reference)	1.00 (reference)
	rs1052133	GG	17 (4.6%)	20 (5.5%)	0.85 (0.44-1.65)	0.92 (0.40-1.84)
	*APEX*1 E148D	CC/CT	289 (78.1%)	262 (72.2%)	1.00 (reference)	1.00 (reference)
	rs3136819	TT	81 (21.9%)	101 (27.8%)	0.68 (0.48-0.97)	0.70 (0.50-0.98)
Direct repair						
DM	*MGMT* F84L	CC	299 (81.0%)	267 (73.6%)	1.00 (reference)	1.00 (reference)
	rs12917	CT/TT	70 (19.0%)	96 (26.4%)	0.67 (0.45-0.95)	0.69 (0.47-0.97)
RM	*MGMT* V143I	AA/AG	379 (97.9%)	369 (98.8%)	1.00 (reference)	1.00 (reference)
	rs2308321	GG	8 (2.1%)	4 (1.2%)	1.95 (0.58-6.84)	N.A
	MGMT R178K	AA/AG	364 (97.8%)	359 (98.7%)	1.00 (reference)	1.00 (reference)
	rs2308327	GG	8 (2.2%)	4 (1.3%)	1.97 (0.57-6.67)	N.A
DSBR						
RM	*XRCC3* T241M	CC/CT	308 (83.5%)	315 (87.5%)	1.00 (reference)	1.00 (reference)
	rs861539	TT	61 (16.5%)	45 (12.5%)	1.43 (0.93-2.18)	1.46 (0.92-2.23)
	NBS Q185E	GG/GC	341 (91.4%)	318 (87.1%)	1.00 (reference)	1.00 (reference)
	rs1805794	CC	32 (8.6%)	47 (12.9%)	0.65 (0.40-1.05)	0.66 (0.40-1.03)
Others						
DM	*LIG1* 5′UTR	CC	285 (76.6%)	255 (69.9%)	1.00 (reference)	1.00 (reference)
	rs20579	CT/TT	87 (23.4%)	110 (30.1%)	0.67 (0.48-0.94)	0.68 (0.48-0.92)
RM	*LIG1* A170A	AA/AC	278 (74.7%)	281 (77.0%)	1.00 (reference)	1.00 (reference)
	rs20580	CC	94 (25.3%)	84 (23.0%)	1.14 (0.81-1.61)	1.16 (0.81-1.61)

NOTE: The Akaike's information criterion was used to determine the genetic model for each single-nucleotide polymorphism.
Abbreviations: SNP, single-nucleotide polymorphism; OR, odds ratio; DM, dominant genetic model; RM, recessive genetic model; N.A, not available; NER, nucleotide excision repair; BER, base excision repair; DSBR, DSB repair.
*Adjusted for age and gender.
† Bootstrapping mean odds ratio and its corresponding 95% CI.

glioma, which included oligodendroglioma, not-otherwise-specified astrocytoma, and mixed glioma.

Individual Single-Nucleotide Polymorphism Association Analysis. The single-nucleotide polymorphism identifications, locations, and allele frequencies are given in Supplementary Table S1. The genotype distributions of 18 selected single-nucleotide polymorphisms in the cases and controls are summarized in Table 2. All genotype distributions in the controls were consistent with those expected from the Hardy-Weinberg equilibrium test (Supplementary Table S1). The allele frequencies of three single-nucleotide polymorphisms (that is, *MGMT* F84L, *LIG1* 5′UTR, and *XRCC3* T241M) were significantly different between the cases and the controls, even after 1,000 permutations ($P = 0.028$, 0.044, and 0.044, respectively). Marginal differences in distribution were observed for *XPD* Q751K, *XRCC1* R399Q, and *PARP1* A762V after 1,000 permutations ($P = 0.059$, 0.066, and 0.064, respectively).

Logistic regression analyses revealed that in the dominant-effect model, as assessed by the Akaike's information criterion, compared with wild-type homozygote carriers, significant increased risk effects were associated with *XRCC1* R399Q (adjusted odds ratio, 1.43; 95% CI, 1.05-1.92), whereas significantly protective effects were associated with *PARP1* A762V (adjusted odds ratio, 0.71; 95% CI, 0.52-0.97), *MGMT* F84L (adjusted odds ratio, 0.66; 95% CI, 0.45-0.95), and *LIG1* 5′UTR (adjusted odds ratio, 0.67; 95% CI, 0.48-0.94). Meanwhile, in the recessive-effect model (assuming that only the variant homozygotes have an increased risk for glioma) compared with the wild-type homozygotes and heterozygous carriers, a significantly increased risk

Table 3. Stratified analysis by age, gender, ionizing radiation exposure history, and histologic type of glioma

Variables	Dominant								Recessive			
	XRCC1 R399Q		*PARP1* A762V		*MGMT* F84L		*LIG1* 5′UTR		*ERCC1* 3′UTR		*APEX1* E148D	
	OR (95% CI)*		OR (95% CI)*		OR (95% CI)*		OR (95% CI)*		OR (95% CI)*		OR (95% CI)*	
	No.†	GA/AA vs GG	No.†	TC/CC vs TT	No.†	CT/TT vs CC	No.†	CT/TT vs CC	No.†	GG vs TT/GT	No.†	TT vs CC/CT
Age (y)												
≤45	142/143	1.47 (0.91-2.38)	142/143	0.62 (0.38-1.06)	140/143	0.57 (0.32-1.00)	142/143	0.46 (0.27-0.80)	142/143	5.88 (1.64-20.0)	142/143	0.96 (0.57-1.64)
>45	231/221	1.39 (0.95-2.03)	230/221	0.76 (0.52-1.14)	230/220	0.76 (0.49-1.20)	230/221	0.85 (0.55-1.29)	230/220	1.08 (0.52-2.27)	230/220	0.54 (0.36-0.85)
P for interaction		0.86		0.54		0.44		0.085		0.016		0.11
Gender												
Male	214/159	0.94 (0.62-1.44)	213/159	0.58 (0.38-0.91)	211/159	0.53 (0.32-0.88)	213/159	0.79 (0.50-1.24)	214/157	1.92 (0.78-4.76)	213/158	0.70 (0.43-1.14)
Female	158/204	2.27 (1.45-3.57)	158/205	0.86 (0.55-1.35)	156/203	0.86 (0.53-1.40)	158/205	0.56 (0.34-0.92)	156/204	1.79 (0.76-4.15)	156/204	0.67 (0.42-1.10)
P for interaction		0.0047		0.23		0.19		0.31		0.90		0.91
IR exposure history												
No	333/335	1.37 (1.00-1.89)	332/336	0.76 (0.55-1.06)	329/335	0.68 (0.48-1.00)	332/336	0.71 (0.50-1.01)	331/334	1.89 (1.00-3.56)	331/334	0.70 (0.49-1.01)
Yes	35/21	3.45 (1.11-11.1)	35/21	0.20 (0.08-0.62)	35/21	0.76 (0.20-2.94)	35/21	0.26 (0.08-0.91)	35/20	1.32 (0.11-16.7)	34/21	0.40 (0.10-1.54)
P for interaction		0.14		0.036		0.82		0.072		0.79		0.44
Histologic type												
High grade	213/363	1.46 (1.01-2.08)	212/364	0.79 (0.54-1.15)	211/362	0.70 (0.46-1.08)	212/364	0.77 (0.51-1.14)	214/361	1.97 (1.00-3.95)	212/362	0.50 (0.32-0.78)
Medium grade	76/363	1.15 (0.69-1.90)	76/364	0.52 (0.28-0.95)	74/362	0.52 (0.26-0.99)	76/364	0.47 (0.24-0.88)	75/361	1.77 (0.66-4.71)	76/362	1.32 (0.75-2.30)
Low grade	82/363	1.51 (0.91-2.51)	82/364	0.72 (0.41-1.22)	82/364	0.75 (0.42-1.35)	82/364	0.66 (0.38-1.17)	80/361	1.66 (0.63-4.37)	79/362	0.91 (0.51-1.63)

*Adjusted for age and gender, accordingly.
† Numbers of cases/controls.

Table 4. Haplotype analyses of glioma risk

Gene	Haplotype	Frequency			OR (95% CI)*	Global score test †
		Total	Cases	Controls		
XPC						0.281
	C C	0.394	0.387	0.401	1.00 (reference)	
	C A	0.370	0.387	0.353	1.16 (0.92-1.48)	
	T A	0.235	0.225	0.246	0.95 (0.72-1.23)	
XPD						0.025
	T T	0.423	0.422	0.429	1.00 (reference)	
	G G	0.340	0.370	0.307	1.20 (0.94-1.54)	
	G T	0.212	0.193	0.232	0.86 (0.65-1.15)	
	T G	0.025	0.015	0.032	0.40 (0.17-0.98)	
XRCC1						0.071
	C T	0.563	0.542	0.584	1.00 (reference)	
	C A	0.360	0.383	0.336	1.28 (1.03-1.59)	
	T T	0.077	0.074	0.079	1.05 (0.66-1.69)	
MGMT						0.280
	C A A	0.774	0.788	0.759	1.00 (reference)	
	T A A	0.113	0.097	0.128	0.77 (0.54-1.08)	
	C G G	0.103	0.107	0.099	1.09 (0.76-1.54)	
	T G G	0.010	0.007	0.013	0.56 (0.11-2.78)	
LIG1						0.120
	C C	0.476	0.485	0.466	1.00 (reference)	
	C A	0.382	0.391	0.372	1.03 (0.82-1.31)	
	T A	0.123	0.112	0.135	0.75 (0.53-1.08)	
	T C	0.019	0.012	0.027	0.51 (0.17-1.49)	

NOTE: Loci chosen for *XPC*, W499R and K939Q; *XPD*, R156R and Q751K; *XRCC1*, W194I and R399Q; *MGMT*, F84L, V143I, and R178K; *LIG1*, 5′UTR and A170A.
*Adjusted for age and gender.
† Generated by permutation test with 1,000 times.

was associated with the variant homozygotes of *ERCC1* 3′UTR (adjusted odds ratio, 1.86; 95% CI, 1.01-3.46), whereas a significant protective effect was associated with the variant homozygotes of *APEX1* E148D (adjusted odds ratio, 0.68; 95% CI, 0.48-0.97). All bootstrap odds ratios were very similar to the presented adjusted odds ratios (Table 2).

Further, we stratified our analyses by gender, age, ionizing radiation exposure history, and glioma histologic type on the aforementioned six single-nucleotide polymorphisms, that is, the four protective single-nucleotide polymorphisms (*PARP1* A762V, *MGMT* F84L, *LIG1* 5′UTR, and *APEX1* E148D) and the two risk single-nucleotide polymorphisms (*ERCC1* 3′UTR and *XRCC1* R399Q). As shown in Table 3, the protective effect of *PARP1* A762V (adjusted odds ratio, 0.52; 95% CI, 0.28-0.95), *MGMT* F84L (odds ratio, 0.52; 95% CI, 0.26-0.99), and *LIG1* 5′UTR (odds ratio, 0.47; 95% CI, 0.24-0.88) were more evident in patients with medium-grade tumors, whereas the *APEX1* E148D variant was more evident in patients with high-grade tumors (adjusted odds ratio, 0.50; 95% CI, 0.32-0.78). In addition, the effect of *PARP1* A762V variants was more significant in the subjects exposed to ionizing radiation (adjusted odds ratio, 0.20; 95% CI, 0.08-0.62; $P_{interaction}$ = 0.036). For the risk-effect single-nucleotide polymorphisms, the increased risk effect of *XRCC1* R399Q was more evident in females (adjusted odds ratio, 2.27; 95% CI, 1.45-3.57; $P_{interaction}$ = 0.0047), whereas the *ERCC1* 3′UTR variant was more pronounced in younger cases (adjusted

Table 5. Cumulative genetic risk analysis of adverse genotypes in glioma cases and control subjects

No. of variant or adverse genotypes	No. of cases/controls	OR (95% CI)	*P* for trend
The total six risk-conferring SNPs			0.0004
0 ~ 2*	45/70	1.00 (reference)	
3 ~ 4	223/232	1.55 (1.00-2.35)	
5 ~ 6	93/55	2.67 (1.59-4.57)	
IR exposure and the total six risk-conferring SNPs			0.008
No IR and 0 ~ 2 †	44/62	1.00 (reference)	
No IR and 3 ~ 6	280/269	1.45 (0.91-2.25)	
Have IR and 0 ~ 2	9/12	1.08 (0.40-2.97)	
Have IR and 3 ~ 6	24/8	4.28 (1.65-11.41)	

NOTE: Adjusted for age and gender.
*We treat the minor allele of the two-risk-effect single-nucleotide polymorphisms and the common allele of the four-protective-effect single-nucleotide polymorphisms as the adverse allele, and set individuals with fewer than three adverse alleles as the reference group. Adverse genotypes: for four protective single-nucleotide polymorphisms, *APEX1* E148D (recessive, V V), *PARP* A762V (dominant, V V + W V), *MGMT* F84L (dominant, V V + W V), and *LIG1* 5′UTR (dominant, V V + W V); for the two risk single-nucleotide polymorphisms, *ERCC1* 3′UTR (recessive, W W + W V) and *XRCC1* R399Q (dominant, W W).
† The no ionizing radiation exposure group and with fewer than three adverse genotypes defined as the reference.

odds ratio, 5.88; 95% CI, 1.64-20.0; $P_{interaction}$ = 0.016). However, both the *XRCC1* R399Q (adjusted odds ratio, 1.46; 95% CI, 1.01-2.08) and *ERCC1* 3′UTR (odds ratio, 1.97; 95% CI, 1.00-3.95) variants were more pronounced in patients with high-grade gliomas (Table 3).

Haplotype Analysis. Strong linkage disequilibrium was observed between each pair of the single-nucleotide polymorphisms in the *XPC*, *XPD*, *XRCC1*, and *LIG1* genes (data not shown). Table 4 summarizes the associations between the frequency distributions of the haplotypes and the risk for glioma. For *XPD*, haplotype ''TG'' showed the greatest protective effect, accounting for a 60% reduction in the risk for glioma (adjusted odds ratio, 0.40; 95% CI, 0.17-0.98). For *XRCC1*, one risk haplotype ''CA'' was identified (adjusted odds ratio, 1.28; 95% CI, 1.03-1.59). No haplotypes of the *XPC*, *MGMT*, and *LIG1* genes were found to be significantly associated with glioma risk or protection. Furthermore, the global score test showed a statistically significant difference in the haplotype distributions between cases and controls for *XPD* (global P = 0.025) and a borderline association with *XRCC1* (global P = 0.071).

Gene-Gene and Gene-Environment Interactions Analysis

Cumulative Genetic Risk Test of Multiple Single-Nucleotide Polymorphism Association. To test our hypothesis that multiple single-nucleotide polymorphisms in the DNA repair pathways act together to modulate glioma risk, we estimated the global effect of the six adverse single-nucleotide polymorphisms that were significantly associated with glioma risk in the single-locus analysis. Two risk-effect single-nucleotide polymorphisms and four protective-effect single-nucleotide polymorphisms were included in this analysis; because they exhibited risk in opposite directions, we treated the minor allele of the risk single-nucleotide polymorphisms and the common allele of the protective single-nucleotide polymorphisms as the adverse allele, and regarded individuals with fewer than three adverse alleles as the reference group. As shown in Table 5, the risk for glioma increased progressively as the number of adverse genotypes increased (P_{trend} = 0.0004). That is, the groups with three to four and five to six adverse genotypes all exhibited a significantly increased glioma risk. Then, we further stratified our analyses by ionizing radiation exposure. Likewise, a significant dose-response effect on glioma risk was observed (P_{trend} = 0.008). Specifically, compared with the reference group (individuals with fewer than three adverse alleles who had no ionizing radiation exposure), the non-ionizing radiation exposure groups with three to six adverse genotypes only showed a marginal significantly increased glioma risk (adjusted odds ratio, 1.45; 95% CI, 0.91-2.25), whereas the ionizing radiation exposure groups with three to six adverse genotypes showed an odds ratio of 4.28 (95% CI, 1.65-11.41). These data showed a significant association when interactions among the six single-nucleotide polymorphisms and ionizing radiation were considered. However, the results from this cumulative genetic risk analyses should be interpreted with caution because of

A

No. factor	Best candidate model	Prediction error (%)	*P*	CVC
1	*MGMT* F84L	52.75	0.9824	81/100
2	*MGMT* F84L, *APEX1* E148D	52.79	0.9557	61/100
3	*PARP1* A762V, *MGMT* F84L, *APEX1* E148D	50.33	0.8159	74/100
4	**IR exposure, *PARP1* A762V, *MGMT* F84L, *APEX1* E148D**	**44.38**	**0.0443**	**100/100**
5	IR exposure, *PARP1* A762V, *MGMT* F84L, *APEX1* E148D, *ERCC1* 3′UTR	47.21	0.0967	100/100

B

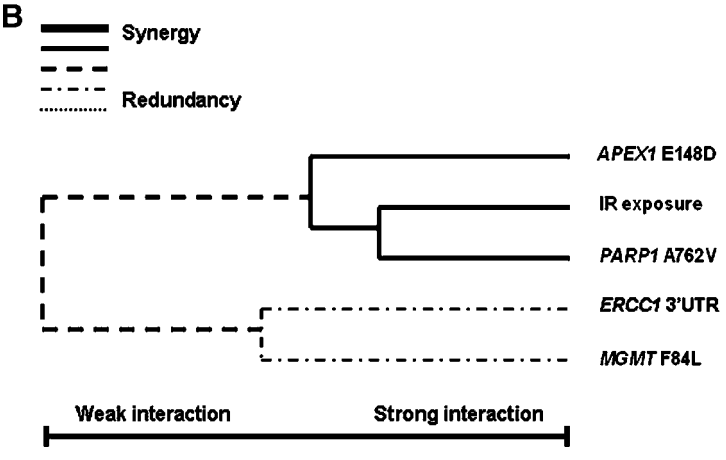

Figure 1. The multifactor dimensionality reduction models and interaction dendrogram for gene-gene and gene-environment interactions on glioma risk. **A.** Summary of the multifactor dimensionality reduction interaction models. CVC, cross-validation consistency. *P* based on 1,000 permutation. **B.** Interaction dendrogram. The attributes (single-nucleotide polymorphism or ionizing radiation) that strongly interact to appear close together at the leaves of the tree, with those not interacting appearing distant from one another.

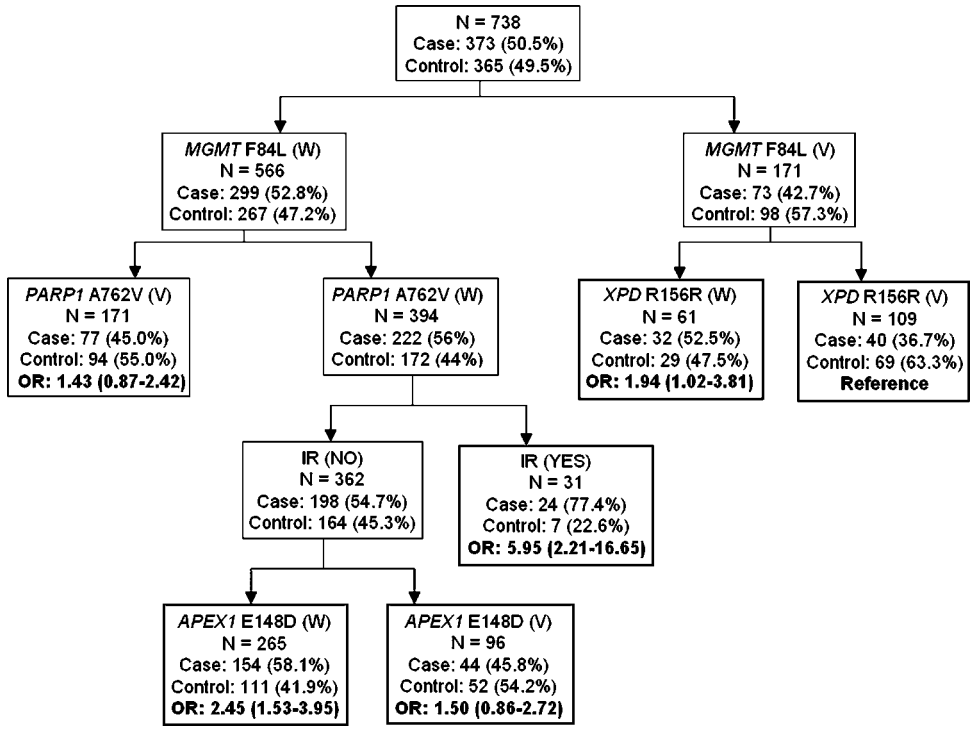

Figure 2. Classification and regression tree analysis of the DNA repair gene polymorphisms and ionizing radiation exposure history. The number and percentage of cases and controls are shown for each node. W, wild-type genotypes; V, variant genotypes. Reference group is the least percentage of cases. Odds ratio and 95% CI were adjusted by age and gender.

the small size of the subgroup and the borderline confidence intervals.

Multifactor Dimensionality Reduction Analysis. Figure 1A summarizes the best interaction models obtained from the multifactor dimensionality reduction analysis. The best one-locus model for predicting glioma risk was *MGMT* F84L (testing accuracy, 47.25%; cross-validation consistency, 81; permutation $P = 0.982$). But the best interaction model was a four-locus model (that is, ionizing radiation, *PARP1* A762V, *MGMT* F84L, and *APEX1* E148D), with an improved testing accuracy of 55.62% (cross-validation consistency, 100; permutation $P = 0.044$). The five-locus model (that is, ionizing radiation, *PARP1* A762V, *MGMT* F84L, *APEX1* E148D, and *ERCC1* 3′UTR) also had an improved testing accuracy of 52.89% and 100 cross-validation consistency. However, it only had borderline interactions with an empirical $P = 0.096$ based on 1,000 permutations.

The multifactor dimensionality reduction analysis indicated that the four-locus model was the best model but did not specify the presence of synergy between the loci. To test this possibility, we applied the interaction dendrogram to determine their relationship. Figure 1B illustrates the interaction dendrogram for these models. Consistent with the stratified analysis, the hierarchical cluster analysis placed ionizing radiation exposure and *PARP1* A762V on the same branch, which shows that the strongest interactions exist between them.

Classification and Regression Tree Analysis. Figure 2 depicts the resulting tree structure generated by the classification and regression tree analysis. Consistent with the multifactor dimensionality reduction one-locus model, the first split on the decision tree was *MGMT* F84L, confirming that this single-nucleotide polymorphism was the most important risk factor for glioma among those considered. Further inspection of the classification and regression tree structure suggested distinct patterns for the wild-type (W) and the variant (V) alleles of *MGMT* F84L. In particular, individuals with the combined polymorphisms of *MGMT* F84L (V) and *XPD* R156R (V) exhibited the lowest glioma risk, with a 36.7% case rate. Using this terminal node as the reference group, the individuals who had ionizing radiation exposure exhibited the highest glioma risk in the setting of both the *MGMT* F84L and *PARP1* A762V wild-type genotypes (adjusted odds ratio, 5.95; 95% CI, 2.21-16.65), further supporting the strong interaction between *PARP* A762V and ionizing radiation exposure shown by the interaction dendrogram. In addition, the subgroups with *MGMT* F84L (W), *PARP1* A762V (W), no ionizing radiation exposure, and *APEX1* E148D (W) also had an increased glioma risk (adjusted odds ratio, 2.45; 95% CI, 1.53-3.95). However, these results are based on small numbers and should be interpreted with care.

Discussion

In this study, we used multianalytic strategies to systematically examine the associations between a panel of DNA repair genes single-nucleotide polymorphisms and glioma risk. In the single-locus analysis, six single-nucleotide polymorphisms showed a significant association with glioma risk. When evaluating the cumulative genetic risk of these six single-nucleotide polymorphisms, we found a significant trend toward increased risk with an increasing number of adverse genotypes. Moreover, the multifactor dimensionality reduction and classification and regression tree analyses identified *MGMT* F84L as the predominant risk factor for glioma

and revealed strong interactions among ionizing radiation exposure, *PARP1* A762V, *MGMT* F84L, and *APEX1* E148D. Taken together, these consistent findings imply that gene-gene, gene-environment interactions contribute to glioma susceptibility. However, a definite conclusion can only be reached on external validation by larger studies.

Perhaps the most significant finding in this study was the consistent association of *MGMT* F84L with glioma risk, which was identified using different analytic approaches. In the single-locus analysis, *MGMT* F84L had the strongest allelic association with glioma susceptibility, and the genotypic analysis also revealed that it was strongly associated with glioma risk. Consistent with the single-locus association results, the best one-factor model in multifactor dimensionality reduction and the first split in classification and regression tree both identified *MGMT* F84L as the predominant risk factor for glioma. Thus, this significant association was consistent across all analyses, which suggests that our results are unlikely due to chance but may reveal a biological link between *MGMT* F84L and the etiology of glioma.

Although the functional relevance of the *MGMT* F84L is unknown, several lines of evidence suggest that this finding is biologically plausible. As a pivotal gene involved in repair of DNA lesions induced by alkylating oxidative agents, *MGMT* is located on chromosome 10q26 and can act alone to reverse alkylation damage (32). Studies have suggested that the region of chromosome 10 (10q24-q26) is one of the commonly deleted regions in glioma and likely to contain the tumor suppressor gene important in glioma tumor formation (33). Moreover, *MGMT* F84L was previously reported to be associated with risk for endometrial cancer (34) and glioma (7, 35). A recently published paper, Bethke et al. (12) found that *MGMT* F84L was marginally significant in five unique glioma case-control series from four different countries (1,013 cases, 1,016 controls). Han et al. (36) showed that the association between breast cancer and the F84L may be magnified by an increased endogenous estrogen level. Another colorectal cancer study reported that significant interactions were found between this single-nucleotide polymorphism and alcohol intake and body mass index (37). These studies lend support for a yet unexplained association between environment factors and *MGMT* F84L for cancer risk. Bugni et al. (38) suggested that gene-environment interactions may be particularly important for *MGMT* because its effect on cancer susceptibility in experimental animals is strongly determined by exposure to alkylating agents. Although *MGMT* is unlikely to be directly related to ionizing radiation, it may depend on its interaction with other genes in the same pathway, such as p53. It is reported that p53 is involved in regulation of the *MGMT* by DNA damaging agents such as alkylating agents, ionizing radiation, and UV light (39). Further mechanistic studies are therefore warranted to address the functional relevance of *MGMT* F84Lvariant.

It is also important to note that of the six single-nucleotide polymorphisms associated with glioma risk, three (*XRCC1* R399Q, *PARP1* A762V, and *APEX*1 E148D) were from the base excision repair pathway, suggesting a strong link between base excision repair and glioma. It has been suggested that base excision repair plays a critical role in the maintenance of the central nervous system genomic integrity (40), and recent studies show that base excision repair is active in neuronal cells in culture, in brain cells in experimental animal models and in human postmortem brain tissue (41). Considering that the most important culprit for causing DNA damage in the brain seems to be oxidative stress and base excision repair is the most important DNA repair pathway to deal with such damage in the brain (42), it is therefore very likely that the single-nucleotide polymorphisms in the base excision repair could play an important role in glioma development.

Several studies have found an increased risk for brain tumors after exposure to high doses of ionizing radiation such as atomic bomb survivors (2, 43, 44); however, results after exposure to chronic lower doses are not consistent. Although a possible association between low-level medical ionizing radiation exposure and the risk for brain tumors has been suggested (45, 46), occupational exposure to low ionizing radiation levels and the risk for brain tumors remains, but not very consistent (45, 47, 48). In the current study, 10 study subjects reported medical exposure to ionizing radiation (for treatment of conditions such as acne, birth mark, and hyperthyroidism), whereas the remaining 46 were occupationally exposed (occupations such as physicians, radiologists, nurses, pilots, and engineers). Our stratified analysis and interaction dendrogram model detected a gene-ionizing radiation interaction for glioma risk that could potentially be explained by altered protein function resulting from the *PARP1* A762V variants, thus leading to suboptimal repair of DNA damage. Given that ionizing radiation is one of the most important risk factors for glioma; our results may have implication on future studies.

Given that our study population is relatively small, several approaches were taken to control for false-positive findings. First, we included only Caucasian cases in the analysis. This ethnic homogeneity of study population reduces the risk of confounding by unmeasured factors, either genetic or environmental. Second, most of the genes and single-nucleotide polymorphisms selected were based on previous biological evidence of their considerable functional importance. Under these circumstances, the frequency of false positive findings would be substantially decreased and the power of association would be improved by accounting for their interactions. Last, we used two different strategies to assess the robustness of associations: an internal validation procedure based on bootstrap resampling methods (that is, using the same data set) and a correction for multiple testing using permutation tests. Two major advantages of bootstrapping are that it makes no assumptions about the distribution of the data and that it gives more accurate answers as a result of correction for small sample sizes (24).

Despite the strengths and biological plausibility of the associations observed in our study, there are inherent limitations. A major limitation is the small number of participants with ionizing radiation exposure in the analyses. Because the ionizing radiation exposure assessment was based on self-reported information, the possibility of recall bias or misclassification due to subjects not being fully aware of their ionizing radiation exposures, especially in occupational settings, exists. Quantitative studies on low-dose ionizing radiation

exposure in the general population are very difficult to conduct because large samples and long-term complete follow-up are needed in addition to lifetime measures of doses. Several previous studies examined the association between DNA repair gene polymorphisms and cancer risk, but none examined interactions of polymorphisms with ionizing radiation exposures, except for studies on populations exposed specifically to medical ionizing radiation (49, 50). Another limitation is the limited power to examine interactions. Given the *post hoc* data-driven nature of the classification and regression tree approach, the small sample sizes in some of the terminal nodes, and the wide confidence intervals led to the generation of findings that should be cautiously interpreted. However, these findings suggest new directions for the examination of gene-gene and gene-environment interactions for future studies.

In summary, our study suggested that several polymorphisms in DNA repair genes may act individually or together to contribute to glioma susceptibility. The mechanism of how the variants modify the effect of ionizing radiation needs further elucidated through experimental studies. In particular, our results support the notion that future risk assessments of complex diseases such as cancer need to move from the analysis of single polymorphisms to the systematic verification of the gene-environment-disease network.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Association and Interactions between DNA Repair Gene Polymorphisms and Adult Glioma

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