Associations Between *ERCC2* Polymorphisms and Gliomas[1]

Michele Caggana,[2] Jennifer Kilgallen, James M. Conroy, John K. Wiencke, Karl T. Kelsey, Rei Miike, Pengchin Chen, and Margaret R. Wrensch

Division of Genetic Disorders, New York State Department of Health, Wadsworth Center, Albany, New York 12201 [M. C., J. K., J. M. C.]; Department of Epidemiology and Biostatistics, School of Medicine, University of California, San Francisco, San Francisco, California 94143 [J. K. W., R. M., P. C., M. R. W.]; and Department of Cancer Biology, Department of Environmental Health, Harvard School of Public Health, Boston, Massachusetts 02115 [K. T. K.]

Abstract

Xeroderma pigmentosum complementation group D/excision repair cross-complementing in rodents 2 (*ERCC2*) encodes a protein that is part of the nucleotide excision repair pathway and the transcription factor IIH transcription complex. Mutations in this gene have been shown to cause three distinct clinical diseases including xeroderma pigmentosum, Cockayne syndrome, and trichothiodystrophy. Several *ERCC2* polymorphisms, the effects of which on gene function are not known, have been described. To investigate whether constitutive sequence variations might be associated with adult onset gliomas, blood specimens from a case-control study (187 cases and 169 controls) were genotyped for seven previously described polymorphisms (R156R, I199M, H201Y, D312N, A575A, D711D, and K751Q). A novel R616C polymorphism was also identified. Cases were significantly more likely than controls to be homozygous for the silent AA variant at codon 156 (odds ratio, 2.3; 95% confidence interval, 1.3–4.2). Although this was observed for patients in each of three histological subgroups of cases, (glioblastoma multiforme, astrocytoma, and oligoastrocytoma) compared with controls, the association was strongest for patients with oligoastrocytoma (odds ratio, 3.2; 95% confidence interval, 1.1–9.5). In contrast, cases were somewhat less likely than controls to carry variants at D312N, D711D, and K751Q, but not significantly so overall or for any subgroup after adjustment for age and gender. Individuals with variant nucleotides at D312N, D711D, and K751Q were significantly more likely to carry a variant at another of those three codons and less likely to carry a variant nucleotide at R156R, regardless of case or control status. Although the pattern of association observed here is consistent with a role of *ERCC2* variants in the prevention or causation of glioma, these results are also consistent with the possibility that another gene linked to *ERCC2* may be involved. This seems especially so because the strongest association was observed with a silent nucleotide variation.

Introduction

Several rare cancer-prone syndromes caused by highly penetrant single gene defects have been described, including Li-Fraumeni and Bloom syndromes and XP.[3] Diverse environmental factors have been implicated in epidemiological studies of glioma. These include radiation, chemicals, and viral or infectious exposures, but only therapeutic radiation appears to be a generally accepted risk factor (1). Certain rare genetic syndromes (*e.g.*, neurofibromatosis, tuberous sclerosis, and Li-Fraumeni family cancer syndromes) are known to predispose to glioma, but collectively these account for a small proportion of cases (2, 3). Because substantial genetic heterogeneity exists even within tumors of the same histological subtype, it is generally believed that there are multiple pathways of genetic alterations leading to glioma (4). In addition, the same chromosomal region that harbors the *ERCC2* gene and other NER proteins was found to be altered by gain and loss of 19q13.2–13.3 in a study of familial gliomas (5, 6) and nonfamilial gliomas (7). Recently, a study (8) showed that loss of 19q and 1p was significantly related to the overall survival of patients specifically with oligodendroglioma but not with astrocytomas, and loss of heterozygosity of 19q13.3 occurred most frequently in oligodendrogliomas (73% in the sample set; Ref. 6). Studies (9) on glioma cell lines have revealed translocations in the 19q13 region. Thus, an important glioma gene(s) may reside in the same region as *ERCC2*.

Patients with XP exhibit an increased sensitivity to sunlight and are prone to skin cancer from UV-induced DNA damage. Individuals with XP are also at risk for other cancers that result from unrepaired damage (10), including brain cancer (11). The studies of Dabholkar *et al.* (12) and Liang *et al.* (13) showed different tissue expression patterns and alterations in genomic copy number for *ERCC2* in malignant brain tumors when compared with nonmalignant brain tissue. Both studies postulated that because of its role in correcting transcription-coupled DNA damage, the ERCC2 helicase of the DNA NER pathway may be involved in glial cell tumorigenesis.

Several different complementation groups have been characterized in these patients. The XP complementation group D (*XPD*)/*ERCC2* (14, 15) gene functions as a helicase (16) in the NER pathway and transcription initiation by binding to the transcription factor IIH via p44 (17). Whereas ERCC2 protein

Received 5/17/00; revised 1/3/01; accepted 1/16/01.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
[1] Supported by CA52689 and CA57220 from National Cancer Institute, by ES04705 and ES06717 from National Institute of Environmental Health Sciences, by IRG-97-150-01-IRG from the American Cancer Society, by the United States Environmental Protection Agency, and also by the New York State Department of Health.
[2] To whom requests for reprints should be addressed, at the Division of Genetic Disorders, Wadsworth Center, New York State Department of Health, P.O. Box 509, Albany, NY 12201-0509.

[3] The abbreviations used are: XP, xeroderma pigmentosum; *ERCC2*, excision repair cross-complementing in rodents 2; NER, nucleotide excision repair; SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval.

Table 1 Amplification and detection parameters for *ERCC2* polymorphism detection

Primer name	Primer sequence	Annealing temperature	Detection (polymorphism, gene location)[a]
XPD6F	5′ TGG AGT GCT ATG GCA CGA TCT CT 3′	55°C	*Tfi I* digestion (**R156R**, C to A, exon 6)
XPD6R	5′ CCA TGG GCA TCA AAT TCC TGG GA 3′		
XPD811F	5′ GGC CTG TGT GGG AGT GAC GG 3′	59°C	*BstYI* digestion (**I199M**, C to G, exon 8), *SphI* digestion (**H201Y**, C to T, exon 8) N-5′ GTG CTG CCC GAC GAA GT 3′[b] M-5′ ACT TCG TTG GGC AGC AC 3′[b] (**D312N**, G to A, exon 10)
XPD811R	5′ GGA CAC GGC TCT GCA TAA CC 3′		
XPD1819F	5′ CTG TGC CCT GAA CCC ACC CTG 3′	55°C	*BanI* digestion (**A575A**, C to T, exon 18)
XPD1819R	5′ AGA GAG CTC TGG GAA GAC ACC 3′		
XPD2021F	5′ CCC CAA CTC AGA CAC AGC AT 3′	57°C	*BsaHI* digestion (**R616C**, C to T, exon 20)
XPD2021R	5′ CGC ATG GCA TCG AAG GTA AG 3′		
XPD2122F	5′ GGC TGT TTC CCG TTC ATT TC 3′	59°C	N-5′ ACC GTG GAC GAG GGT GT 3′[b] M-5′ ACA CCC TCA TCC ACG GT 3′[b] (**D711D**, C to T, exon 22)
XPD2122R	5′ GTA GAT GCA CGA TAA ACT TC 3′		
XPD23F	5′ CCC CCT CTC CCT TTC CTC TG 3′	59°C	*MboII* digestion (**K751Q**, A to C, exon 23)
XPD23R	5′ AAC CAG GGC CAG GCA AGA C3′		

[a] Bold, mutation names.
[b] Detection via allele-specific oligonucleotide hybridization.

restores NER in deficient cells (18), it does not seem to be required for *in vivo* transcription (19) but may enhance it (20). ERCC2 protein defects are also responsible for two additional (21) clinically distinct diseases including trichothiodystrophy and Cockayne syndrome. Individuals with trichothiodystrophy suffer from mental retardation, ichthyosis, brittle hair, and dwarfism. Cockayne syndrome patients with ERCC2 defects have XP-type neoplasia plus retinal degeneration, mental retardation, dwarfism, and hyperreflexia.

In addition to studies identifying disease-causing mutations in XP (17, 22–26), sequence analysis of the *ERCC2* (GenBank accession no. L47234) gene revealed several DNA polymorphisms (27, 28). It is possible that genetic alterations in *ERCC2* result in subtle effects on the efficiency of the NER pathway and may be involved in damage caused by environmental factors, including oxidative damage. To investigate potential relationships between the *ERCC2* sequence alterations and their role in the etiology of gliomas, several polymorphisms were studied in a series of patients with glioma and controls to determine their prevalence in these populations.

Materials and Methods

Study Population. The study population has been described previously (29, 30). Cases and controls for the study were drawn from the San Francisco Bay Area adult glioma study and discussed in detail elsewhere (3). Incident glioma cases (n = 492; age >20 years) were ascertained from August 1991 to April 1994 in six San Francisco Bay Area counties through the rapid case ascertainment service of the Northern California Cancer Center. Uniform neuropathology review indicated 4 cases were not glioma, and specimens could not be reviewed for 12 subjects. Thus, the parent study included 476 cases. A random digit-dialing technique was used to contact 462 controls, and these samples were frequency matched to the cases for gender, ethnicity, and age (3). Blood specimens were collected part of the way through the study (29, 30), and thus samples available for genotyping included 187 cases with pathology review and 171 controls. Of these, 161 cases and 164 controls were white. The protocol was reviewed and approved by the Committee for Human Subject Research at the University of California, San Francisco.

DNA Isolation and Genotyping. DNA was isolated from heparinized whole blood or tumor tissue using Chelex or Qiagen column purification. DNA was insufficient for any PCR amplification for two controls. The amplification primers for each polymorphism are given in Table 1. Each reaction included 0.2–0.6 μM primers mixed with 50 ng of genomic DNA, 1.0–2.0 mM $MgCl_2$, 0.8 units of Taq DNA polymerase, and 200 μM deoxynucleotide triphosphates in 10 mM Tris, 50 mM KCl (pH 8.3) in a final reaction volume of 50 μl. Amplification was carried out by initial denaturation at 95°C for 5 min followed by 35 cycles of denaturation at 95°C for 15 s, annealing at 57–59°C for 15 s, and extension at 72°C for 30 s. A final extension step was carried out for 5 min at 72°C. Restriction digestion or allele-specific oligonucleotide hybridization was used to detect the polymorphisms (Table 1). Digestion products were electrophoresed for 30 min at 150 V on 3% NuSieve:1% LE agarose (FMC Biochemicals) and stained with ethidium bromide (10 μg/ml). Hybridizations were carried out using tetramethylammonium chloride at 52°C using [γ-^{32}P]ATP-labeled probes.

Statistical Analyses. Genotype and allele frequencies among cases and controls were compared, and χ^2 tests were conducted to assess similarity of genotype frequencies. In addition, genotype frequencies were compared in three histological subgroups of cases for which there were sufficient numbers for separate analyses [glioblastoma multiforme, astrocytoma (including anaplastic astrocytoma), and oligoastrocytoma]. Next, the frequencies of all of the cases, case by histological subgroup (n = 3 subgroups), and controls were computed for each polymorphism. This analysis was done by comparing one *versus* no variant alleles first and then by comparing two *versus* one or no variant alleles. Logistic regression was used to estimate unadjusted, age-adjusted, and gender-adjusted ORs for having the indicated genotype in all of the cases *versus* controls and then in each histological category *versus* controls. Kendall correlations were used to assess associations among *ERCC2* genotypes. Wilcoxon tests and ANOVA were used to compare median and mean ages of subjects with different genotypes, respectively. Goodness of fit to Hardy-Weinberg equilibrium expectations was assessed by χ^2 test for each polymorphism.

Results

Table 2 shows the demographics of the overall population, the population genotyped, and whites genotyped. There was a higher proportion of men among genotyped subjects than among the parent population. Genotyped cases were signifi-

Table 2 Comparison of the study group included in *ERCC2* genotype analysis to the overall study population of brain tumor cases and controls in the San Francisco Bay Area Adult Glioma Study, 1991–1995

	Total study population		All of the genotyped subjects		Whites genotyped	
	Cases (n = 476)	Controls (n = 462)	Cases (n = 187)	Controls (n = 169)	Cases (n = 161)	Controls (n = 162)
Gender						
Female (%)	204 (43%)	209 (45%)	69 (37%)	79 (47%)	63 (39%)	75 (46%)
Male (%)	272 (57%)	253 (55%)	118 (63%)	90 (53%)	98 (61%)	87 (54%)
Age (yr) (Mean ± SE)	54.2 ± 0.8	53.7 ± 0.8	48.3 ± 1.1	53.0 ± 1.2	48.1 ± 1.2	53.3 ± 1.2
Diagnosis by cell type: (%)[a]						
Glioblastoma multiforme	281 (59%)		82 (44%)		67 (42%)	
Astrocytoma[b]	89 (19%)		39 (21%)		34 (21%)	
Oligoastrocytoma	47 (10%)		31 (17%)		28 (17%)	
Others	59 (12%)		35 (19%)		32 (20%)	
Race (white) (%)	400 (84%)	397 (86%)	161 (86%)	162 (96%)	161 (100%)	162 (100%)

[a] Percentages shown are percentage of total tumors.
[b] Includes anaplastic astrocytoma.

cantly younger (P = 0.003) than the overall case population (48.3 ± 1.1 years *versus* 54.2 ± 0.8 years) and less likely to have aggressive tumors (*i.e.*, glioblastoma) than those in the parent study (44% *versus* 59%, respectively). This is most likely because of the decreased survival of older individuals with more aggressive tumors during the lag time between diagnosis, ascertainment, and follow-up venipuncture. Because the preponderance of genotyped study participants (86% of cases and 96% of controls) were white, the results presented here are restricted to this group.

A total of eight polymorphisms were studied (Table 1). Three were silent changes that did not result in amino acid substitutions. These included R156R, A575A, and D711D. The remaining five nucleotide changes included: I199M, H201Y, D312N, R616C, and K751Q. The variant allele frequencies for I199M, H201Y, A575A, and R616C were very low (none detected, 0.7%, 0.5%, and 0.4%, respectively) in this population and were excluded from additional statistical analyses. Genotype and allele frequencies for the remaining four variants are given in Table 3. The genotype frequencies of each polymorphism appeared to be in Hardy-Weinberg equilibrium in all of the controls. Among cases, the R156R (χ^2 = 7.4; P = 0.01), D312N (χ^2 = 4.7; P = 0.03), and D711D (χ^2 = 4.1; P = 0.04) polymorphisms appeared not to be in Hardy-Weinberg equilibrium. Analyses in histological subgroups of cases revealed significant differences from Hardy-Weinberg expectations for polymorphisms in R156R in subjects with either glioblastoma or oligoastrocytomas, in D312N in subjects with glioblastoma, and in D711D in patients with astrocytoma. Discrepancies in the numbers of study subjects shown in Table 3 and Table 4 (see below) compared with the summary (Table 2) were because of technical difficulties with the amplification of some of the DNA samples.

Because the R616P mutation resulted in a null allele (30) and was not found to be causative for XP, it was chosen originally for inclusion in this study. A BsaHI RFLP assay was designed to detect the R616P variant (see Table 1). The consensus for the restriction endonuclease is 5′ G[A,G]CG[C,T]C 3′. When a detected positive sample was sequenced for confirmation, the expected CGT to CCT change was not identified, but rather a CGT to TGT change was observed resulting in the rare, newly identified R616C polymorphism. Previously, two other sequence variations, R616P and a double mutant, R616W (CGT to TGG), have been reported (22, 24).

Cases were more likely than controls to have the variant nucleotide at R156R (P = 0.07) but were less likely than controls to carry variant nucleotides at D312N, D711D, and K751Q. χ^2 tests for each of three genotypes in subgroups of patients *versus* controls revealed that patients with oligoastrocytomas had a significantly different distribution of genotypes than controls at codon positions R156R (P = 0.0002), D312N (P = 0.03), and D711D (P = 0.01).

Cases were significantly more likely than controls to be homozygous variant AA at R156R, regardless of histology (see Table 4; age- and gender-adjusted OR, 2.3; 95% CI, 1.3–4.2). The association was strongest for patients with oligoastrocytoma *versus* controls (see Table 4). Cases were less likely than controls to carry a variant nucleotide at each of the codons D312N, D711D, and K751Q (see Table 4), but none of the differences achieved statistical significance after adjustment for age and gender. Within each category of subjects, cases and controls, median or mean ages of people with different genotypes did not differ significantly.

Variants at D312N, D711D, and K751Q were highly significantly correlated with each other and were each strongly negatively correlated with the presence of the variant nucleotide at R156R (Table 5). That is, an individual carrying a variant at D312N, D711D, and K751Q was more likely to carry the variant at the others and less likely to carry the variant at R165R. This was true regardless of case status. The positive correlation was particularly striking between D711D and K751Q, which are only 605 bases apart on the chromosome. The smallest correlation was observed for the two polymorphisms in which the genetic distance is greatest (R156R and K751Q; 13.4 kb).

Discussion

The evolving field of SNP analysis is becoming more and more widely used for association studies to map and study genetic variation (31–33). Several recent studies (34–36) have shown that in many instances it is not appropriate to determine the frequency of one given SNP to determine its association with a complex disease. The effects of a given polymorphism considered separately may be hampered by haplotype differences and linkage disequilibrium (36). The study reported here shows that when eight different polymorphisms are studied in the *ERCC2* gene, one variant is more frequently associated with cases. However, all of the remaining variant alleles (three of which occurred with sufficient frequency for study) occur at higher frequencies in the control population, raising the possibility that these three additional variants may be inversely associated.

Table 3 Frequencies of *ERCC2* genotypes in white brain tumor patients and controls, stratified by tumor histopathology in the San Francisco Bay Area Adult Glioma Study, 1991–1995

	Genotype-R156R			Allele frequencies	
	CC[a]	CA[a]	AA[a]	C (%)	A (%)
Controls (n = 139)	55 (40)	60 (43)	24 (17)	61.1	38.8
All of the Cases (n = 126)	38 (30)	49 (39)	39 (31)	49.6	50.4
Glioblastoma multiforme (n = 48)	14 (29)	17 (35)	17 (35)	46.9	53.1
Astrocytoma (n = 29)	8 (28)	12 (41)	9 (31)	48.3	51.7
Oligoastrocytoma (n = 22)	8 (36)	6 (27)	8 (36)	50.0	50.0
Others (n = 27)	8 (30)	14 (52)	5 (19)	55.6	44.4
	Genotype-D312N			Allele frequencies	
	GG[a]	GA[a]	AA[a]	G (%)	A (%)
Controls (n = 137)	56 (41)	64 (47)	17 (12)	64.2	35.8
All of the Cases (n = 135)	67 (50)	51 (38)	17 (13)	59.7	27.4
Glioblastoma multiforme (n = 51)	28 (55)	15 (29)	8 (16)	69.6	30.4
Astrocytoma (n = 29)	13 (45)	12 (41)	4 (14)	65.5	34.4
Oligoastrocytoma (n = 26)	16 (62)	8 (31)	2 (8)	76.9	23.1
Others (n = 29)	10 (34)	16 (55)	3 (10)	62.1	37.9
	Genotype-D711D			Allele frequencies	
	CC[a]	CT[a]	TT[a]	C (%)	T (%)
Controls (n = 140)	65 (46)	59 (42)	16 (11)	67.5	32.5
All of the Cases (n = 114)	60 (53)	39 (34)	15 (13)	69.7	30.3
Glioblastoma multiforme (n = 45)	24 (53)	16 (36)	5 (11)	71.1	28.9
Astrocytoma (n = 24)	14 (58)	6 (25)	4 (17)	70.8	29.2
Oligoastrocytoma (n = 19)	13 (68)	5 (26)	1 (5)	81.6	18.4
Others (n = 26)	9 (35)	12 (46)	5 (19)	57.7	42.3
	Genotype-K751Q			Allele frequencies	
	AA[a]	AC[a]	CC[a]	A (%)	C (%)
Controls (n = 148)	49 (33)	76 (51)	23 (16)	58.8	41.2
All of the Cases (n = 148)	62 (42)	63 (43)	23 (16)	63.2	36.8
Glioblastoma multiforme (n = 63)	28 (44)	26 (41)	9 (14)	65.1	34.9
Astrocytoma (n = 32)	10 (31)	17 (53)	5 (16)	57.8	42.2
Oligoastrocytoma (n = 25)	13 (52)	9 (36)	3 (12)	70.0	30.0
Others (n = 28)	11 (39)	11 (39)	6 (21)	58.9	41.1

[a] Number in group (%).

These variants are also positively correlated in the population, irrespective of case status, such that carriers of any one of these are more likely to carry the others. The utility of SNP data varies depending on the goals of the study (31, 34) because linkage disequilibrium may occur at very large distances in some instances and small distances in other genomic regions. Thus, data examining multiple SNPs in a gene become difficult to analyze, unless haplotypes can be established.

Observed significant ORs for the R156R variant for the overall case group (OR, 2.3) ranging to (OR, 3.2) for cases with oligoastrocytoma are somewhat surprising, because this variant most associated with case-control status is a silent polymorphism in exon 6 of the gene. Dybdahl *et al.* (37) also found that the AA variant was associated with basal cell carcinomas in psoriatics. The significance of these findings will have to await functional analysis of this as well as the other polymorphisms. Additionally, in this same series of gliomas, Chen *et al.* (38) studied an A to C polymorphism in the 3′ untranslated region of the *ERCC1* gene, which curiously resides in the coding region of a nucleolar gene on the opposite strand (*ASE-1/CAST*). *ERCC2* and *ERCC1* reside in the 19q region, raising the possibility that multiple polymorphisms in multiple NER genes increase the risk of disease. An alternative explanation is that both of these genes are in linkage disequilibrium with another nearby gene. One such potential candidate, the human glia maturation factor-γ gene, is located on chromosome 19q13.2. Mutational analysis of this gene did not reveal any sequence variations in glioma patients, whether or not they demonstrated loss of heterozygosity (39). It is also known that the kinesin light chain (*KLC2*) gene lies tail to tail with *ERCC2* (40).

The different frequencies of the R156R polymorphism among glioma cases and controls could be attributable to either an influence of *ERCC2* or a nearby gene on gliomagenesis, progression, or survival from glioma. The latter possibilities are suggested by the fact that this polymorphism is a silent nucleotide variation and by the observation that it and two other polymorphisms were not in Hardy-Weinberg equilibrium. Despite using a population-based rapid case ascertainment program that on average identified cases within 2 months of diagnosis, 44% of cases were deceased by the time of study contact because of the very poor survival associated with glioblastoma. This required interviews with proxies and precluded specimen collection for such subjects. The problem was further compounded because we did not begin blood collection until part of the way through the study. Studies in which specimens for constitutive DNA analyses are collected at the time of diagnosis will be necessary to distinguish whether *ERCC2* or a nearby gene

Table 4 Numbers and median ages of *ERCC2* genotypes in white tumor patients and controls, stratified by tumor histopathology in the San Francisco Bay Area Adult Glioma Study, 1991–1995

R156R (C to A)	Genotypes and median ages		ORs	
	No. CA/CC (Median age)[a]	No. AA (Median age)	OR (95% CI)	Age/gender adjusted OR (95% CI)
Controls	115 (52)	24 (64)	1.0[b]	1.0[b]
All of the cases	87 (47)	39 (48)	2.1 (1.2–3.8)	2.3 (1.3–4.2)
Glioblastoma multiforme	31 (58)	17 (63)	2.6 (1.3–5.5)	2.4 (1.1–5.0)
Astrocytoma	20 (41)	9 (49)	2.2 (0.9–5.3)	2.8 (1.1–7.4)
Oligoastrocytoma	14 (40)	8 (33)	2.7 (1.0–7.2)	3.2 (1.1–9.5)
D312N (G to A)	No. GG (Median age)[a]	No. AG/AA (Median age)	OR (95% CI)	Age/gender adjusted OR (95% CI)
Controls	56 (54)	81 (52)	1.0[b]	1.0[b]
All of the cases	67 (48)	68 (46)	0.7 (0.4–1.1)	0.7 (0.5–1.2)
Glioblastoma multiforme	28 (60)	23 (56)	0.6 (0.3–1.1)	0.6 (0.3–1.1)
Astrocytoma	13 (41)	16 (43)	0.9 (0.4–1.9)	1.0 (0.4–2.2)
Oligoastrocytoma	16 (36)	10 (36)	0.4 (0.2–1.0)	0.5 (0.2–1.3)
D711D (C to T)	No. CC (Median age)[a]	No. CT/TT (Median age)	OR (95% CI)	Age/gender adjusted OR (95% CI)
Controls	65 (54)	75 (53)	1.0[b]	1.0[b]
All of the cases	60 (48)	54 (47)	0.8 (0.5–1.3)	0.8 (0.5–1.3)
Glioblastoma multiforme	24 (60)	21 (57)	0.8 (0.4–1.5)	0.8 (0.4–1.6)
Astrocytoma	14 (46)	10 (39)	0.6 (0.3–1.5)	0.6 (0.2–1.4)
Oligoastrocytoma	13 (32)	6 (41)	0.4 (0.1–1.1)	0.4 (0.2–1.3)
K751Q (A to C)	No. AA (Median age)[a]	No. AC/CC (Median age)	OR (95% CI)	Age/gender adjusted OR (95% CI)
Controls	49 (52)	99 (52)	1.0[b]	1.0[b]
All of the cases	62 (49)	86 (48)	0.7 (0.4–1.1)	0.7 (0.4–1.1)
Glioblastoma multiforme	28 (57)	35 (58)	0.6 (0.3–1.1)	0.6 (0.3–1.1)
Astrocytoma	10 (50)	22 (39)	1.1 (0.5–2.5)	1.1 (0.5–2.5)
Oligoastrocytoma	13 (32)	12 (41)	0.5 (0.2–1.1)	0.5 (0.2–1.3)

[a] Age is age at diagnosis for cases and at interview for controls.
[b] Referent group.

Table 5 Kendall correlation coefficients and *P*s for white cases and controls in the San Francisco Bay Area Adult Glioma Study, 1991–1995

	Correlation (*P*)			
	R156R	D312N	D711D	K751Q
R156R				
D312N	−0.44 (0.0001)			
D711D	−0.42 (0.0001)	0.54 (0.0001)		
K751Q	−0.32 (0.0001)	0.38 (0.0001)	0.78 (0.0001)	
Any rare variant	0.12 (0.04)	−0.03 (0.61)	0.005 (0.94)	0.02 (0.70)

is involved in tumor formation or progression and treatment response. Another potential weakness of our study was that our response rate among controls in the parent study was only 63%, but it seems unlikely that *ERCC2* genotype would influence control participation. This study had 77% power to detect the observed 2.1-fold OR for the R156R variant at the 0.05 level of significance but substantially less power for the weaker associations observed for the more prevalent variants at the other loci. Additional studies of these polymorphisms in other populations will be necessary to confirm these results, and larger sample sizes will be needed to examine potentially relevant gene-environment interactions.

Acknowledgments

We thank the Molecular Genetics Core at the Wadsworth Center for oligonucleotide synthesis and sequence analysis. We thank Salvatore Duva for expert technical assistance.

References

1. Wrensch, M. R., Bondy, M. L., Wiencke, J., and Yost, M. Environmental risk factors for primary malignant brain tumors: a review. J. Neuro-oncol., *17:* 47–64, 1993.

2. Bondy, M., Wiencke, J., Wrensch, M., and Kyritsis, A. P. Genetics of primary brain tumors: a review. J. Neuro-oncol., *18:* 69–81, 1994.

3. Wrensch, M., Lee, M., Miike, R., Newman, B., Barger, G., Davis, R., Wiencke, J., and Neuhaus, J. Familial and personal medical history of cancer and nervous system conditions among adults with glioma and controls. Am. J. Epidemiol., *145:* 581–593, 1997.

4. Rasheed, B. K., Wiltshire, R. N., Bigner, S. H., and Bigner, D. D. Molecular pathogenesis of malignant gliomas. Curr. Opin. Oncol., *11:* 162–167, 1999.

5. Patel, A., van Meyel, D. J., Mohapatra, G., Bollen, A., Wrensch, M., Cairncross, J. G., and Feuerstein, B. G. Gliomas in families: chromosomal analysis by comparative genomic hybridization. Cancer Genet. Cytogenet., *100:* 77–83, 1998.

6. Smith, J. S., Alderete, B., Minn, Y., Borell, T. J., Perry, A., Mohapatra, G., Hosek, S. M., Kimmel, D., O'Fallon, J., Yates, A., Feuerstein, B. G., Burger, P. C., Scheithauer, B. W., and Jenkins, R. B. Localization of common deletion regions on 1p and 19q in human gliomas and their association with histological subtype. Oncogene, *18:* 4144–4152, 1999.

7. Rubio, M-P., Correa, K. M., Ueki, K., Mohrenweiser, H. W., Gusella, J. F., von Deimling, A., and Louis, D. N. The putative glioma tumor suppressor gene on chromosome 19q maps between APOC2 and HRC. Cancer Res., *54:* 4760–4763, 1994.

8. Smith, J. S., Perry, A., Borell, T. J., Lee, H. K., O'Fallon, J., Hosek, S. M., Kimmel, D., Yates, A., Burger, P. C., Scheithauer, B. W., and Jenkins, R. B. Alterations of chromosome arms 1p and 19q as predictors of survival in oligodendrogliomas, astrocytomas, and mixed oligoastrocytomas. J. Clin. Oncol., *18:* 636–645, 2000.

9. Chernova, O., and Cowell, J. K. Molecular definition of chromosome translocations involving 10q24 and 19q13 in human malignant glioma cells. Cancer Genet. Cytogenet., *105:* 60–68, 1998.

10. Giglia, G., Bouffet, E., Jouvet, A., Ohgaki, H., Kleihues, P., and Sarasin, A. Molecular analysis of glioma and skin-tumour alterations in a xeroderma-pigmentosum child. Int. J. Cancer, *81:* 345–350, 1999.

11. Kraemer, K. H., Lee, M. M., and Scotto, J. DNA repair protects against cutaneous and internal neoplasia: evidence from xeroderma pigmentosum. Carcinogenesis (Lond.), *5*: 511–514, 1984.

12. Dabholkar, M. D., Berger, M. S., Vionnet, J. A., Egwuagu, C., Silber, J. R., Yu, J. J., and Reed, E. Malignant and nonmalignant brain tissues differ in their messenger RNA expression patterns for *ERCC1* and *ERCC2*. Cancer Res., *55:* 1261–1266, 1995.

13. Liang, B. C., Ross, D. A., and Reed, E. Genomic copy number changes of DNA repair genes *ERCC1* and *ERCC2* in human gliomas. J. Neuro-oncol., *26:* 17–23, 1995.

14. Weber, C. A., Salazar, E. P., Stewart, S. A., and Thompson, L. H. Molecular cloning and biological characterization of a human gene, *ERCC2*, that corrects the nucleotide excision repair defect in CHO UV5 cells. Mol. Cell. Biol., *8:* 1137–1146, 1988.

15. Weber, C. A., Salazar, E. P., Stewart, S. A., and Thompson, L. H. *ERCC2*: cDNA cloning and molecular characterization of a human nucleotide excision repair gene with high homology to yeast RAD3. EMBO J., *9:* 1437–1447, 1990.

16. Sung, P., Bailly, V., Weber, C., Thompson, L. H., Prakash, L., and Prakash, S. Human xeroderma pigmentosum group D gene encodes a DNA helicase. Nature (Lond.), *365:* 852–855, 1993.

17. Coin, F., Marinoni, J-F., Rodolfo, C., Fribourg, S., Pedrini, A. M., and Egly, J-M. Mutations in the XPD helicase gene result in XP and TTD phenotypes, preventing interaction between XPD and the p44 subunit of TFIIH. Nat. Genet., *20:* 184–188, 1998.

18. Quilliet, X., Chevallier-Lagente, O., Eveno, E., Stojkovic, T., Destee, A., Sarasin, A., and Mezzina, M. Long-term complementation of DNA repair deficient human primary fibroblasts by retroviral transduction of the *XPD* gene. Mutat. Res., *364:* 161–169, 1996.

19. Winkler, G. S., Araujo, S. J., Fiedler, U., Vermeulen, W., Coin, F., Egly, J-M., Hoeijmakers, J. H. J., Wood, R. D., Timmers, H. T. M., and Weeda, G. TFIIH with inactive XPD helicase functions in transcription initiation but is defective in DNA repair. J. Biol. Chem., *275:* 4258–4266, 2000.

20. Tirode, F., Busso, D., Coin, F., and Egly, J-M. Reconstitution of the transcription factor TFIIH: assignment of functions for the three enzymatic subunits, XPB, XPD, and cdk7. Mol. Cell, *3:* 87–95, 1999.

21. Bootsma, D., Kraemer, K. H., Cleaver, J. E., and Hoeijmakers, J. H. J. Nucleotide excision repair syndromes: xeroderma pigmentosum, Cockayne syndrome, and trichothiodystrophy. *In:* C. R. Scriver, A. L. Beaudet, W. S. Sly, and D. Valle (eds.), The Metabolic and Molecular Bases of Inherited Disease, pp. 245–274. New York: McGraw-Hill, Inc., 1998.

22. Taylor, E. M., Broughton, B. C., Botta, E., Stefanini, M., Jaspers, N. G., Fawcett, H., Harcourt, S. A., Arlett, C. F., and Lehmann, A. R. Xeroderma pigmentosum and trichothiodystrophy are associated with different mutations in the *XPD* (*ERCC2*) repair/transcription gene. Proc. Natl. Acad. Sci. USA, *94:* 8658–8663, 1997.

23. Frederick, G. D., Amirkhan, R. H., Schultz, R. A., and Friedberg, E. C. Structural and mutational analysis of the xeroderma pigmentosum group D (*XPD*) gene. Hum. Mol. Genet., *3:* 1783–1788, 1994.

24. Broughton, B. C., Steingrimsdottir, H., Weber, C., and Lehmann, A. R. Mutations in the xeroderma pigmentosum group D DNA repair/transcription gene in patients with trichothiodystrophy. Nat. Genet., *7:* 189–194, 1994.

25. Kobayashi, T., Kuraoka, I., Saijo, M., Nakatsu, Y., Tanaka, A., Someda, Y., Fukuro, S., and Tanaka, K. Mutations in the *XPD* gene leading to xeroderma pigmentosum symptoms. Hum. Mutat., *9:* 322–331, 1997.

26. Takayama, K., Danks, D. M., Salazar, E. P., Cleaver, J. E., and Weber, C. A. DNA repair characteristics and mutations in the *ERCC2* DNA repair and transcription gene in a trichothiodystrophy patient. Hum. Mutat., *9:* 519–525, 1997.

27. Shen, M. R., Jones, I. M., and Mohrenweiser, H. Nonconservative amino acid substitution variants exist at polymorphic frequency in DNA repair genes in healthy humans. Cancer Res., *58:* 604–608, 1998.

28. Broughton, B. C., Steingrimsdottir, H., and Lehmann, A. R. Five polymorphisms in the coding sequence of xeroderma pigmentosum group D gene. Mutat. Res., *362:* 209–211, 1996.

29. Wiencke, J. K., Wrensch, M. R., Miike, R., Zuo, Z-F., and Kelsey, K. T. Population-based study of glutathionine *S*-transferase μ gene deletion in adult glioma cases and controls. Carcinogenesis (Lond.), *18:* 1431–1433, 1997.

30. Kelsey, K. T., Wrensch, M., Zuo, Z-F., Miike, R., and Wiencke, J. K. A population-based case-control study of the CYP2D6 and GSTT1 polymorphisms and malignant brain tumors. Pharmacogenetics, *7:* 463–468, 1997.

31. Collins, F. S., Brooks, L. D., and Chakravarti, A. A DNA polymorphism discovery resource for research on human genetic variation. Genome Res., *8:* 1229–1231, 1998.

32. Brookes, A. J. The essence of SNPs. Gene (Amst.), *234:* 177–186, 1999.

33. Nickerson, D. A., Taylor, S. L., Weiss, K. M., Clark, A. G., Hutchinson, R. G., Stengard, J., Salomaa, V., Vartiainen, E., Boerwinkle, E., and Sing, C. DNA sequence diversity in a 9.7-kb region of the human lipoprotein lipase gene. Nat. Genet., *19:* 233–240, 1998.

34. Kruglyak, L. Prospects of whole-genome linkage disequilibrium mapping of common disease genes. Nat. Genet., *22:* 139–144, 1999.

35. Risch, N., and Merikangas, K. The future of genetic studies of complex diseases. Science (Washington DC), *273:* 1516–1517, 1996.

36. Ober, C., Leavitt, S. A., Tsalenko, A., Howard, T. D., Hoki, D. M., Daniel, R., Newman, D. L., Wu, X., Parry, R., Lester, L. A., Solway, J., Blumenthal, M., King, R. A., Xu, J., Meyers, D. A., Bleecker, E. R., and Cox, N. J. Variation in the interleukin 4-receptor a gene confers susceptibility to asthma and atopy in ethnically diverse populations. Am. J. Hum. Genet., *66:* 517–526, 2000.

37. Dybdahl, M., Vogel, U., Frentz, G., Wallin, H., and Nexo, B. A. Polymorphisms in the DNA repair gene *XPD*: correlations with risk and age at onset of basal cell carcinoma. Cancer Epidemiol. Biomark. Prev., *8:* 77–81, 1999.

38. Chen, P., Wiencke, J., Aldape, K., Diaz, A., Miike, R., Kelsey, K., Lee, M., Liu, J., and Wrensch, M. Association of an ERCC1 polymorphism with adult onset glioma. Cancer Epidemiol. Biomark. Prev., *9:* 843–847, 2000.

39. Peters, N., Smith, J. S., Tachibana, I., Lee, H. K., Pohl, U., Portier, B. P., Louis, D. N., and Jenkins, R. B. The human glia maturation factor-γ gene: genomic structure and mutation analysis in gliomas with chromosome 19q loss. Neurogenetics, *2:* 163–166, 1999.

40. Lamerdin, J. E., Stilwagen, S. A., Ramirez, M. H., Stubbs, L., and Carrano, A. V. Sequence analysis of the *ERCC2* gene regions in human, mouse, and hamster reveals three linked genes. Genomics, *34:* 399–409, 1996.