Polymorphisms in Apoptosis and Cell Cycle Control Genes and Risk of Brain Tumors in Adults

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Abstract

Despite the potential importance of the cell cycle and apoptosis pathways in brain tumor etiology, little has been published regarding brain tumor risk associated with common gene variants in these pathways. Using data from a hospital-based case-control study conducted by the National Cancer Institute between 1994 and 1998, we evaluated risk of glioma (n = 388), meningioma (n = 162), and acoustic neuroma (n = 73) with respect to 12 single nucleotide polymorphisms from 10 genes involved in apoptosis and cell cycle control: *CASP8, CCND1, CCNH, CDKN1A, CDKN2A, CHEK1, CHEK2, MDM2, PTEN*, and *TP53*. We observed significantly decreased risk of meningioma with the *CASP8* Ex14-271A>T variant [odds ratio (OR_{AT}, 0.8; 95% confidence interval (95% CI), 0.5-1.2; OR_{AA}, 0.5; 95% CI, 0.3-0.9; P_{trend} = 0.03] and increased risk of meningioma with the *CASP8* Ex13+51G>C variant (OR_{GC}, 1.4; 95% CI, 0.9-2.1; OR_{CC}, 3.6; 95% CI, 1.0-13.1; P_{trend} = 0.04). The CT haplotype of the two *CASP8* polymorphisms was associated with significantly increased risk of meningioma (OR, 1.7; 95% CI, 1.1-2.6), but was not associated with risk of glioma or acoustic neuroma. The *CCND1* Ex4-1G>A variant was associated with increased risk for glioma, and the Ex8+49T>C variant of *CCNH* was associated with increased risk of glioma and acoustic neuroma. The *MDM2* Ex12+162A>G variant was associated with significantly reduced risk of glioma. Our results suggest that common variants in the *CASP8, CCND1, CCNH*, and *MDM2* genes may influence brain tumor risk. Future research in this area should include more detailed coverage of genes in the apoptosis/cell cycle control pathways. (Cancer Epidemiol Biomarkers Prev 2007;16(8):1655–61)

Introduction

The processes of tumor initiation, growth, and proliferation are thought to be largely influenced by genes that regulate vital cellular functions (1). In normal cells, DNA damage is recognized by cellular mechanisms, and responses to prevent the propagation of errors include nucleotide damage repair, activation of checkpoints to arrest the cell cycle, and/or cell apoptosis (2). Hereditary defects in genes that regulate the crucial pathways of cell cycle control and apoptosis have been associated with a number of human malignancies, including tumors of the brain and nervous system (1). The importance of apoptosis and cell cycle control in brain tumor etiology has also been suggested by the frequent presence of mutations or loss of expression of apoptosis/cell cycle control genes such as *PTEN, CDKN2A, MDM2, TP53, NF1*, and *RB* in brain tumors (3).

Despite the potential importance of the cell cycle and apoptosis pathways in brain tumor etiology, very little has been published regarding brain tumor risk associated with more common gene variants in these pathways, with the exception of the *TP53* gene, for which some evidence of differences in glioma and meningioma risk with common polymorphisms and haplotypes has been noted (4-7), and the *CCND* gene, for which no association with meningioma was observed (8). Using data from a hospital-based case-control study conducted by the National Cancer Institute (NCI) between 1994 and 1998, we evaluated risk of three brain tumor types (glioma, meningioma, and acoustic neuroma) with respect to 12 single nucleotide polymorphisms (SNP) from 10 genes involved in apoptosis and cell cycle control: *CASP8* (rs13113, rs1045485), *CCND1* (rs678653, rs603965), *CCNH* (rs2266690), *CDKN1A* (rs1801270), *CDKN2A* (rs3731249), *CHEK1* (rs506504), *CHEK2* (rs2267130), *MDM2* (rs769412), *PTEN* (rs701848), and *TP53*(rs1042522). These polymorphisms were selected for their common occurrence in the population, and for potential functional relevance signaled by nonsynonymous amino acid changes or occurrence in exonic or promoter regions of the gene (Table 1).

Materials and Methods

Study Setting and Population. A detailed description of study methods can be found elsewhere (9). Briefly, subjects for a case-control study of brain tumors were enrolled between 1994 and 1998 from three hospitals located in Phoenix, Arizona; Boston, Massachusetts; and Pittsburgh, Pennsylvania. Each hospital specializes in the treatment of brain tumors and serves as a regional referral center for the diagnosis. The study protocol was approved by the institutional review board of each participating institution, and written informed consent was obtained from each patient or proxy.

Eligible patients were 18 years or older with a first intracranial glioma, meningioma (ICD-O-2 codes 9530-9538), or acoustic neuroma (ICD-O-2 codes 9560) diagnosed at the hospital or during the 8 weeks preceding hospitalization.

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Table 1. Apoptosis and cell cycle genes and SNPs evaluated in the NCI adult brain tumor study

Gene symbol	Gene name	Location	SNP 500 AA or nt variant ID	RS number	Basis of selection	Epidemiologic association	References
					Prevalence*/functional considerations		
CASP8	*Caspase-8, apoptosis-related cysteine peptidase*	2q33-q34	Ex13+51G>C (D285H)	rs1045485	>0.1 prevalence Amino acid change	Breast cancer	(20)
CASP8			Ex14-271A>T	rs13113	>0.1 prevalence Exonic region Selected for haplotype	NHL	(19)
CCND1	*Cyclin D1*	11q13	Ex4-1G>A	rs603965	>0.1 prevalence Affects protein splicing	Bladder cancer, colorectal cancer, head and neck cancer, lung cancer, leukemia, NHL	(24, 25)
CCND1			Ex5+852C>G	rs678653	>0.1 prevalence Exonic region Selected for haplotype		
CCNH	*Cyclin H*	5q13.3-q14	Ex8+49T>C V270A	rs2266690	>0.1 prevalence Amino acid change Gene involved in cell-cycle control		
CDKN1A	*Cyclin-dependent kinase inhibitor 1A*	6p21.2	Ex2+98C>A S31R	rs1801270	>0.1 prevalence Amino acid change Gene alterations in brain tumors		(37)
CDKN2A	*Cyclin-dependent kinase inhibitor 2A*	9p21	Ex3-16G>A A148T	rs3731249	Amino acid change Gene alternations in brain tumors		
CHEK1	*CHK1 checkpoint homologue*	11q24-q24	Ex13+76A>G I471V	rs506504	Exonic region Gene involved in cell-cycle control		
CHEK2	*CHK2 checkpoint homologue*	22q12.1	IVS9-200G>A	rs2267130	>0.1 prevalence Gene involved in cell-cycle control		
MDM2	*Mdm2, transformed 3T3 cell double minute 2, p53 binding protein*	12q14.3-q15	Ex12+162A>G	rs769412	Exonic region Negative regulator of TP53 Gene alterations in brain tumors		(37)
PTEN	*Phosphatase and tensin homologue*	10q23.3	1515 bp 3′ of STP C>T	rs701848	>0.1 prevalence 3′ region often contains regulatory elements important for transcription and translation Gene alterations in brain tumors		(37)
TP53	*Tumor protein p53*	17p13.1	Ex4+119C>G P72R	rs1042522	>0.1 prevalence Amino acid change Gene alterations in brain tumors		(37)

Abbreviation: NHL, non–Hodgkin's lymphoma.
*Based on SNP500.

Ninety-two percent of eligible brain tumor patients agreed to participate, and 489 patients with glioma, 197 with meningioma, and 96 patients with acoustic neuroma were enrolled. All diagnoses of glioma and meningioma and 96% of acoustic neuromas were confirmed by microscopy at the hospital of diagnosis.

Study controls were patients admitted to the same hospitals as cases for a variety of nonneoplastic conditions, including injuries (25%), circulatory system disorders (22%), musculoskeletal disorders (22%), and digestive disorders (12%). Controls were frequency matched in a 1:1 ratio to all brain tumor patients based on age in years (18-29, 30-39, 40-49, 50-59, 60-69, 70-79, and 80-99 years); race/ethnicity (non-Hispanic White, Hispanic, African-American, other); sex (male, female); hospital (Phoenix, Boston, Pittsburgh); and residential proximity to the hospital in miles (0-4, 5-14, 15-29, 30-49, 50 or more). 799 control patients, who represented 86% of all contacted controls, were enrolled.

Blood samples were collected and sent for genotyping for 388 patients with glioma (79%), 162 patients with meningioma (82%), 73 patients with acoustic neuroma (76%), and 553 controls (69%). The main obstacle to obtaining blood samples was subject refusal, with non-participation in the blood draw being higher for controls (24%) than for cases (14%).

Processing of Blood Samples and Genotyping. DNA was extracted from blood samples using a phenol-chloroform method (10), and genotyping was conducted by the NCI Core Genotyping Facility using a medium-throughput TaqMan assay. Sequence data and assay conditions are provided online[9] (11).

Quality control (QC) specimens for the study included 15 to 34 samples from each of three individuals (A, B, C) who were not study participants (QC-A, $n = 34$; QC-B, $n = 20$; QC-C, $n = 15$) and duplicate samples from 89 study subjects. These specimens were collected and processed in a manner similar to study samples and were interspersed among all genotyping assays in a masked fashion. Percentage agreement between the three nonstudy replicates ranged from 97.8% to 100% for all SNPs. Concordance for duplicates was lowest for *CHEK2* IVS9-200G>A (91.8%), but ranged from 97.2% to 100% for the remaining SNPs, with 100% concordance for nine of the 12 SNPs. In addition to QC specimens, each plate of

Table 2. Demographic characteristics in participants with and without genotyping: NCI adult brain tumor study, 1994 to 1998

Characteristic	Glioma* (n = 388)	Meningioma* (n = 162)	Acoustic neuroma* (n = 73)	Controls* (n = 553)	Total, sent for genotyping (n = 1,176)	Total, no. blood samples (n = 405)
Sex						
Male	211 (54.4)	37 (22.8)	26 (35.6)	254 (45.9)	528 (44.9)	194 (47.9)
Female	177 (45.6)	125 (77.2)	47 (64.4)	299 (54.1)	648 (55.1)	211 (52.1)
Race/ethnicity						
White, non-Hispanic	354 (91.2)	133 (82.1)	66 (90.4)	495 (89.5)	1,048 (89.1)	363 (89.6)
Hispanic	20 (5.2)	12 (7.4)	6 (8.2)	39 (7.1)	77 (6.6)	23 (5.7)
Black	7 (1.8)	9 (5.6)	0 (0.0)	11 (2.0)	27 (2.3)	11 (2.7)
Other	7 (1.8)	8 (4.9)	1 (1.4)	8 (1.5)	24 (2.0)	8 (2.0)
Age at interview (y)						
18-29	46 (11.9)	2 (1.2)	3 (4.1)	75 (13.6)	126 (10.7)	40 (9.9)
30-49	143 (36.9)	60 (37.0)	31 (42.5)	225 (40.7)	459 (39.0)	139 (34.2)
50-69	134 (34.5)	73 (45.1)	30 (41.1)	182 (32.9)	419 (35.6)	148 (36.5)
70-90	65 (16.8)	27 (16.7)	9 (12.3)	71 (12.8)	172 (14.6)	78 (19.3)
Educational level †						
<High school	38 (10.1)	21 (13.0)	4 (5.6)	72 (13.3)	135 (11.5)	63 (15.6)
High school graduate	204 (54.4)	102 (63.4)	33 (45.8)	334 (61.9)	673 (57.2)	232 (57.3)
College or professional	133 (35.5)	38 (23.6)	35 (48.6)	134 (24.8)	340 (28.9)	99 (24.4)
Missing	13	13	1	1	28	11

*Characteristics for individuals sent for genotyping.
† Percent based on nonmissing values.

368 specimens included homozygous wild-type, heterozygous, and homozygous variant positive controls and one DNA negative control.

Statistical Analyses. Statistically significant departure from Hardy-Weinberg equilibrium for controls was assessed using the χ^2 test. For each polymorphism, unconditional logistic regression was used to calculate odds ratios (OR) and 95% confidence intervals (95% CI) for each tumor type, adjusted for the study matching factors of age, sex, race/ethnicity, hospital, and residential proximity to the hospital. Because controls were frequency matched to all tumor types, all controls were used in the models for each tumor type. Models were run under the assumption of codominant inheritance (AA versus Aa versus aa) and dominant inheritance (AA versus Aa or aa). A score test of linear trend was conducted for each SNP using a three-level ordinal variable. To account for the fact some of our results could be false-positive findings due to chance, we calculated the probability that our findings were false positives using the false discovery rate, which reflects the expected ratio of false-positive findings to the number of significant findings (12). We applied the false discovery rate method to the P values for trend as this allows for the fewest number of comparisons and thus degrees of freedom to assess the additive model of risk. For evaluating the false discovery rate, all sets of P_{trend} (glioma, meningioma, and acoustic neuroma) were evaluated simultaneously.

All analyses were run separately by gender and were repeated in non-Hispanic whites (89% of the study population). Given that the protein MDM2 is a strong down-regulator of TP53, we also assessed potential interaction between polymorphisms in these two genes.

Haplotypes formed by the two *CASP8* polymorphisms (D' = 1.0, r^2 = 0.12) and the two *CCND1* polymorphisms (D' = 0.89, r^2 = 0.34) were analyzed within non-Hispanic whites. The HaploView program, version 3.32, was used to estimate haplotype block structure in controls. Haplotype frequencies were estimated using the estimation-maximization algorithm (13), and overall differences between cases and controls (adjusted for age and sex) were assessed using the global score test in HaploStat (R version 2.2.0). The effects of individual haplotypes were estimated using unconditional logistic regression, assuming an additive model and using posterior probabilities of the haplotypes as weights to update the regression coefficients in an iterative manner (14).

Results

Blood samples were available for 1,176 of the 1,581 participants in the brain case-control study. The genotyping success rate for the 12 SNPs ranged from 93.9% to 99.1%. Analysis of all SNPs in the control population did not detect deviation from Hardy-Weinberg equilibrium. Individuals with less education, and those in the oldest age group (70-90 years), were less likely to have samples sent for genotyping (Table 2). Otherwise, the distribution of demographic characteristics for genotyped subjects was similar to the distribution for all study subjects. Relative to controls, subjects with glioma were proportionately more often male, whereas subjects with meningioma and acoustic neuroma were more often female. A lower proportion of subjects with meningioma and acoustic neuroma were in the youngest age bracket (<18 years) compared with controls.

Table 3 summarizes the main effects of the 12 candidate SNPs involved in cell cycle control and apoptosis, for each brain tumor type. We observed no significant association between genotype and risk of any brain tumor (glioma, meningioma, or acoustic neuroma) for the following SNPs: *CCND1* Ex5+852C>G (rs678653), *CDKN1A* Ex2+98C>A (rs1801270), *CDKN2A* Ex3-16G>A (rs3731249), *CHEK1* Ex13+76A>G (rs506504), *CHEK2* IVS9-200G>A (rs2267130), *PTEN* 1,515 bp 3′of STP C>T (rs701848), or *TP53* Ex4+119C>G (rs1042522).

The Ex14-271A>T (rs13113) and Ex13+51G>C (D285H; rs1045485) polymorphisms in *CASP8* were both associated with risk of meningioma: the AT and AA variants of *CASP8* Ex14-271A>T were associated with significantly decreased risk of meningioma (OR_{AT}, 0.8; 95% CI, 0.5, 1.2 and OR_{AA}, 0.5; 95% CI, 0.3-0.9; P_{trend} = 0.03), whereas the GC and CC variants of *CASP8* Ex13+51G>C were associated with increased risk of meningioma (OR_{GC}, 1.4; 95% CI, 0.9, 2.1 and OR_{CC}, 3.6; 95% CI, 1.0, 13.1; P_{trend} = 0.04) as well as acoustic neuroma (OR_{GC}, 1.5; 95% CI, 0.8, 2.6 and OR_{CC}, 5.6; 95% CI, 1.2, 26.6; P_{trend} = 0.04). No association was observed between either of the *CASP8* polymorphisms and glioma. The Ex14-271A>T (rs13113) and Ex13+51G>C (rs1045485) polymorphisms of *CASP8* were found to be in linkage disequilibrium (D' = 1.0, r^2 = 0.12). Haplotype analyses indicated that the CT haplotype of the two SNPs was associated with significantly increased risk of

[9] http://snp500cancer.nci.hih.gov

Table 3. ORs for variants of apoptosis/cell cycle–related SNPs in the NCI adult brain tumor study, 1994 to 1998 (models adjusted for study matching factors age, sex, race/ethnicity, study site, and residential proximity to treatment hospital)

Gene polymorphism	Chromosomal location	Genotype	Controls (n = 553)	Glioma (n = 388)		Meningioma (n = 162)		Acoustic neuroma (n = 73)	
			n	n	OR (95% CI)	n	OR (95% CI)	n	OR (95% CI)
CASP8 (rs13113)	2q33-q34	TT	155	129	1.0 (reference)	60	1.0 (reference)	22	1.0 (reference)
		AT	258	166	0.8 (0.6-1.1)	78	0.8 (0.5-1.2)	30	0.8 (0.5-1.6)
		AA	98	75	0.9 (0.6-1.3)	18	0.5 (0.3-0.9)*	17	1.1 (0.6-2.3)
					P_{trend} = 0.4		P_{trend} = 0.03*		P_{trend} = 0.8
		AT or AA			0.8 (0.6-1.1)		0.7 (0.5-1.1)		0.9 (0.5-1.6)
CASP8 (rs1045485)	2q33-q34	GG	426	284	1.0 (reference)	117	1.0 (reference)	50	1.0 (reference)
		GC	118	95	1.2 (0.8-1.6)	38	1.4 (0.9-2.1)	20	1.5 (0.8-2.6)
		CC	6	3	0.7 (0.1-2.8)	5	3.6 (1.0-13.1)*	3	5.6 (1.2-26.6)*
					P_{trend} = 0.6		P_{trend} = 0.04*		P_{trend} = 0.04*
		GC or CC			1.1 (0.8-1.5)		1.5 (1.0-2.3)*		1.6 (0.9-2.8)
CCND1 (rs678653)	11q13	GG	198	138	1.0 (reference)	72	1.0 (reference)	24	1.0 (reference)
		GC	243	188	1.1 (0.8-1.5)	55	0.6 (0.4-1.0)	33	1.2 (0.7-2.1)
		CC	74	46	0.9 (0.6-1.4)	26	0.9 (0.5-1.6)	13	1.5 (0.7-3.3)
					P_{trend} = 1.0		P_{trend} = 0.3		P_{trend} = 0.3
		GC or CC			1.1 (0.8-1.4)		0.7 (0.5-1.0)		1.2 (0.7-2.2)
CCND1 (rs603965)	11q13	GG	183	105	1.0 (reference)	47	1.0 (reference)	25	1.0 (reference)
		AG	245	196	1.4 (1.0-1.9)*	63	1.1 (0.7-1.7)	36	1.2 (0.7-2.1)
		AA	100	73	1.3 (0.8-1.9)	41	1.5 (0.9-2.5)	10	0.7 (0.3-1.6)
					P_{trend} = 0.2		P_{trend} = 0.2		P_{trend} = 0.6
		AG or AA			1.3 (1.0-1.8)*		1.2 (0.8-1.8)		1.0 (0.6-1.8)
CCNH (rs2266690)	5q13.3-q14	TT	345	238	1.0 (reference)	97	1.0 (reference)	38	1.0 (reference)
		CT	166	117	1.0 (0.7-1.3)	52	1.2 (0.8-1.8)	28	1.8 (1.0-3.1)*
		CC	16	21	2.0 (1.0-3.9)*	5	1.1 (0.4-3.2)	4	2.2 (0.6-7.8)
					P_{trend} = 0.2		P_{trend} = 0.5		P_{trend} = 0.02*
		CT or CC			1.1 (0.8-1.4)		1.2 (0.8-1.8)		1.8 (1.1-3.2)*
CDKN1A (rs1801270)	6p21.2	CC	434	316	1.0 (reference)	125	1.0 (reference)	55	1.0 (reference)
		AC	90	50	0.8 (0.6-1.2)	24	0.8 (0.5-1.4)	12	1.1 (0.5-2.2)
		AA	5	8	2.4 (0.7-7.8)	2	0.5 (0.1-3.3)	2	4.8 (0.5-43.4)
					P_{trend} = 0.9		P_{trend} = 0.4		P_{trend} = 0.4
		AC or AA			0.9 (0.6-1.3)		0.8 (0.5-1.4)		1.2 (0.6-2.3)
CDKN2A (rs3731249)	9p21	GG	523	359	1.0 (reference)	154	1.0 (reference)	69	1.0 (reference)
		AG	24	24	1.4 (0.8-2.5)	5	0.7 (0.2-1.9)	4	1.1 (0.3-3.4)
		AA	—	—	—	—	—	—	—
					P_{trend} = 0.3		P_{trend} = 0.5		P_{trend} = 0.9
		AG or AA			1.4 (0.8-2.5)				
CHEK1 (rs506504)	11q24-q24	GG	474	336	1.0 (reference)	143	1.0 (reference)	66	1.0 (reference)
		AG	42	34	1.1 (0.7-1.8)	9	0.8 (0.4-1.7)	6	1.1 (0.4-2.7)
		AA	1	3	4.8 (0.5-47.4)	0	—	0	—
					P_{trend} = 0.3		P_{trend} = 0.5		P_{trend} = 0.9
		AG or AA			1.2 (0.8-1.9)				
CHEK2 (rs2267130)	22q12.1	AA	167	106	1.0 (reference)	48	1.0 (reference)	21	1.0 (reference)
		AG	240	202	1.3 (0.9-1.8)	76	1.4 (0.9-2.2)	31	1.0 (0.5-1.8)
		GG	104	63	0.9 (0.6-1.4)	32	1.2 (0.7-2.2)	15	1.1 (0.5-2.2)
					P_{trend} = 1.0		P_{trend} = 0.4		P_{trend} = 0.9
		AG or GG			1.2 (0.9-1.6)		1.3 (0.9-2.1)		1.0 (0.6-1.8)
MDM2 (rs769412)	12q14.3-q15	AA	463	345	1.0 (reference)	126	1.0 (reference)	64	1.0 (reference)
		AG	70	32	0.6 (0.4-0.9)*	28	1.4 (0.8-2.3)	8	0.9 (0.4-2.0)
		GG	2	1	0.9 (0.1-10.3)	0	—	0	—
					P_{trend} = 0.03*		P_{trend} = 0.3		P_{trend} = 0.7
		AG or GG			0.6 (0.4-0.9)*				0.9 (0.4-2.0)
PTEN (rs701848)	10q23.3	TT	190	138	1.0 (reference)	71	1.0 (reference)	20	1.0 (reference)
		CT	262	184	0.9 (0.7-1.3)	63	0.8 (0.5-1.1)	39	1.4 (0.8-2.5)
		CC	93	57	0.9 (0.6-1.3)	23	0.7 (0.4-1.3)	14	1.5 (0.7-3.2)
					P_{trend} = 0.5		P_{trend} = 0.2		P_{trend} = 0.2
		CT or CC			0.9 (0.7-1.2)		0.8 (0.5-1.1)		1.4 (0.8-2.5)
TP53 (rs1042522)	17p13.1	GG	300	213	1.0 (reference)	82	1.0 (reference)	44	1.0 (reference)
		CG	209	146	1.0 (0.7-1.3)	64	1.1 (0.8-1.7)	24	0.7 (0.4-1.3)
		CC	38	27	1.0 (0.6-1.7)	13	1.1 (0.5-2.3)	5	1.0 (0.3-2.8)
					P_{trend} = 0.9		P_{trend} = 0.6		P_{trend} = 0.4
		CG or CC			1.0 (0.8-1.3)		1.1 (0.8-1.7)		0.8 (0.4-1.3)

* Significant at $P < 0.05$ level.

Table 4. ORs and 95% CIs for the association between common CASP8 haplotypes and risk of glioma, meningioma and acoustic neuroma, NCI adult brain tumor study, 1994 to 1998

Haplotype*	Glioma (n = 354)		Meningioma (n = 133)		Acoustic neuroma (n = 66)		Controls (n = 495)
	%	OR †	%	OR	%	OR	%
G-A	0.44	1.0 (reference)	0.39	1.0 (reference)	0.46	1.0 (reference)	0.46
G-T	0.42	1.0 (0.8-1.3)	0.44	1.3 (0.9-1.7)	0.35	0.8 (0.5-1.3)	0.42
C-T	0.14	1.1 (0.8-1.5)	0.17	1.7 (1.1-2.6)	0.19	1.5 (0.9-2.5)	0.12
Global test omnibus		0.67		0.048		0.07	

*Order of SNPs is CASP8 Ex13+51G>C and CASP8 Ex14-271A>T.
† ORs and global test are adjusted for age and sex. Analyses restricted to non-Hispanic Whites.

meningioma (OR, 1.7; 95% CI, 1.1, 2.64), but not glioma or acoustic neuroma (Table 4).

The Ex8+49T>C variant of *CCNH* (rs2266690) was associated with increased risk of glioma (OR_{CT}, 1.0; 95% CI, 0.7-1.3 and OR_{CC}, 2.0; 95% CI, 1.0-3.9; P_{trend} = 0.2) and acoustic neuroma (OR_{CT}, 1.8; 95% CI, 1.0-3.1 and OR_{CC}, 2.2; 95% CI, 0.6-7.8; P_{trend} = 0.02), but was not associated with increased risk of meningioma. These trends were more strongly apparent in analyses restricted to non-Hispanic whites for both glioma (OR_{CT}, 1.1; 95% CI, 0.8-1.5 and OR_{CC}, 2.3; 95% CI, 1.1-4.7; P_{trend} = 0.07) and acoustic neuroma (OR_{CT}, 2.2; 95% CI, 1.2-3.9 and OR_{CC}, 3.2; 95% CI, 0.9-11.9; P_{trend} = 0.004).

Individuals with the variant allele of the *CCND1* Ex4-1G>A polymorphism (rs603965) also showed some indication of increased risk for glioma ($OR_{AG/GG}$, 1.3; 95% CI, 1.0-1.8). Although the rare C-A haplotype (frequency = 0.01) was associated with decreased risk of glioma (OR, 0.5; 95% CI, 0.3-0.7), the global test was not statistically significant (P = 0.15). No *CCND1* haplotype associations were observed for meningioma or acoustic neuroma. Significantly reduced risk of glioma was observed for individuals with the G variant of the *MDM2* Ex12+162A>G (rs769412) polymorphism ($OR_{AG/GG}$, 0.6; 95% CI, 0.4-0.9). We observed no significant gene-gene interaction between the *TP53* Ex4+119C>G nonsynonymous SNP and the *MDM2* Ex12+162A>G variant (results not shown). Risk estimates from unadjusted analyses were very similar to adjusted risk estimates. ORs for all statistically significant associations remained very similar when restricted to non-Hispanic whites (Table 5). Although gender-specific estimates were not as stable given smaller numbers, results were generally consistent in males and females (Table 6). None of the observed associations passed the false discovery rate criterion of 15% chance of false-positive findings.

Discussion

Although the etiology of brain and central nervous system tumors is largely unknown, several lines of evidence indicate that events that alter cell cycle control and apoptosis pathways could be of importance. Among the few confirmed risk factors for brain tumors are certain rare predisposing genetic syndromes (15-17). At least three of these syndromes, Li-Fraumeni syndrome, neurofibromatosis 1, and retinoblastoma, are characterized by germ line mutations in genes affecting progression through the cell cycle and apoptosis (*TP53, NF1*, and *RB1*, respectively). The importance of apoptosis and cell cycle control in brain tumor etiology is also suggested by mutations or loss of expression of cell cycle/apoptosis genes (*PTEN, CDKN2A, MDM2, TP53*, and *RB*) in brain tumors (3). More generally, these pathways are of importance in other cancers types, including breast, melanoma, and colon (1).

Activation of the caspase (cysteine aspartyl-specific proteases) family of intracellular cysteine proteases is central to the initiation of apoptosis. CASP8 is the apical caspase in the tumor necrosis factor family death receptor pathway (extrinsic pathway) of apoptosis (18). We observed novel associations

Table 5. Adjusted ORs for variants of selected apoptosis/cell cycle–related SNPs in the NCI adult brain tumor study, Non-Hispanic Whites only

Gene polymorphism	Chromosomal location	Genotype	Glioma		Meningioma		Acoustic neuroma	
			n	OR (95% CI)	n	OR (95% CI)	n	OR (95% CI)
CASP8 (rs13113)	2q33-q34	TT	112	1.0 (reference)	45	1.0 (reference)	19	1.0 (reference)
		AT	153	0.8 (0.6-1.1)	66	0.8 (0.5-1.2)	28	0.8 (0.4-1.6)
		AA	71	0.9 (0.6-1.4)	17	0.5 (0.3-1.0)	15	1.1 (0.5-2.3)
				P_{trend} = 0.4		P_{trend} = 0.04*		P_{trend} = 0.9
CASP8 (rs1045485)	2q33-q34	GG	257	1.0 (reference)	91	1.0 (reference)	44	1.0 (reference)
		GC	89	1.1 (0.8-1.6)	35	1.4 (0.9-2.3)	19	1.5 (0.8-2.7)
		CC	3	0.7 (0.2-2.8)	3	4.0 (1.1-14.8)*	3	5.4 (1.2-26.3)*
				P_{trend} = 0.6		P_{trend} = 0.03*		P_{trend} = 0.03*
CCND1 (rs603965)	11q13	GG	93	1.0 (reference)	36	1.0 (reference)	22	1.0 (reference)
		AG	179	1.3 (1.0-1.9)*	54	1.0 (0.6-1.7)	34	1.2 (0.6-2.1)
		AA	69	1.2 (0.8-1.9)	35	1.4 (0.8-2.5)	8	0.6 (0.3-1.5)
				P_{trend} = 0.2		P_{trend} = 0.3		P_{trend} = 0.4
CCNH (rs2266690)	5q13.3-q14	TT	211	1.0 (reference)	75	1.0 (reference)	32	1.0 (reference)
		CT	112	1.1 (0.8-1.5)	46	1.4 (0.9-2.2)	27	2.2 (1.2-3.9)*
		CC	21	2.3 (1.1-4.7)*	5	1.4 (0.5-4.4)	4	3.2 (0.9-11.9)
				P_{trend} = 0.07		P_{trend} = 0.1		P_{trend} = 0.004*
MDM2 (rs769412)	12q14.3-q15	AA	313	1.0 (reference)	106	1.0 (reference)	57	1.0 (reference)
		AG	30	0.6 (0.4-1.0)	20	1.3 (0.7-2.3)	8	1.0 (0.5-2.4)
		GG	1	0.8 (0.1-9.6)	0	—	0	—
				P_{trend} = 0.06		P_{trend} = 0.5		P_{trend} = 0.9

*Models adjusted for study matching factors age, sex, study site, and residential proximity to treatment hospital.

Table 6. Age-adjusted ORs for variants of selected apoptosis/cell cycle–related SNPs in the NCI adult brain tumor study, Non-Hispanic Whites only, by gender

	Genotype	Males		Females	
		n	OR (95% CI)	*n*	OR (95% CI)
Glioma					
CCND1 (rs603965)	GG	54	1.0 (reference)	39	1.0 (reference)
	AG	98	1.3 (0.8-2.0)	81	1.4 (0.8-2.2)
	AA	37	1.6 (0.9-2.9)	32	1.0 (0.6-1.9)
CCNH (rs2266690)	TT	125	1.0 (reference)	86	1.0 (reference)
	CT	53	0.8 (0.5-1.3)	59	1.6 (1.0-2.4)
	CC	12	2.0 (0.7-5.3)	9	2.5 (0.9-7.2)
MDM2 (rs769412)	AA	174	1.0 (reference)	139	1.0 (reference)
	AG	14	0.5 (0.2-0.9)	16	0.8 (0.4-1.6)
	GG	1	—	0	—
Meningioma					
CASP8 (rs13113)	TT	10	1.0 (reference)	35	1.0 (reference)
	AT	14	0.9 (0.4-2.1)	52	0.8 (0.4-1.3)
	AA	4	0.6 (0.2-2.1)	13	0.5 (0.2-1.1)
CASP8 (rs1045485)	GG	22	1.0 (reference)	69	1.0 (reference)
	GC	4	0.5 (0.2-1.6)	31	1.9 (1.1-3.3)
	CC	2	5.6 (0.8-38.9)	3	2.2 (0.4-11.4)
Acoustic neuroma					
CASP8 (rs1045485)	GG	18	1.0 (reference)	26	1.0 (reference)
	GC	5	0.8 (0.3-2.2)	14	2.1 (1.0-4.4)
	CC	0	—	3	8.0 (1.4-46.9)
CCNH (rs2266690)	TT	10	1.0 (reference)	22	1.0 (reference)
	CT	11	2.1 (0.8-5.2)	16	1.6 (0.8-3.3)
	CC	2	4.8 (0.8-30.5)	2	1.8 (0.3-9.4)

between common genetic variants in *CASP8* and brain tumors: Ex14-271A>T was associated with decreased risk of meningioma, whereas Ex13+51G>C (D285H) was associated with increased risk of meningioma and acoustic neuroma. Our haplotype analyses further implicates the chromosomal region indicated by the two *CASP8* SNPs we evaluated; the significantly increased risk of meningioma observed with the CT haplotype suggests that this chromosomal region either harbors or is in linkage disequilibrium with a functional polymorphism that affects meningioma risk. Others have noted decreased risk of non–Hodgkin's lymphoma with the Ex14-271A>T variant (19). Our finding of increased risk for the D285H nonsynonymous SNP is surprising because of its association with decreased breast cancer risk in other studies, including a large international case-control consortium (20-22). Given that the functionality of this particular SNP has not been well characterized, it is premature to speculate on a mechanistic reason for the inverse direction and the possible role of chance cannot be ruled out.

Elevated risks of glioma and acoustic neuroma were observed for several cell cycle gene polymorphisms. Molecular complexes of the cyclin-dependent kinases and their associated cyclins are responsible for sending out signals from the cell cycle clock to responder molecules that effect the transition of the cell through its cycle of growth and division (23). We found evidence of increased risk of glioma with the Ex4-1G>A (G870A) polymorphism of the *CCND1* gene coding for the cyclin D1 protein. Although no prior study has examined brain tumor risk with respect to the *CCND1* G870A variant, increased risk has been observed with the A allele for several cancer sites, including bladder cancer, colorectal cancer, head and neck cancer, lung cancer, leukemia, and non–Hodgkin's lymphoma (24, 25). Laboratory studies indicate that the A870 allele hinders splicing of the CCND1 protein and may cause production of a variant splice product (''transcript b''; ref. 24). However, it is still unclear as to whether transcript b production is directly associated with the G/A 870 polymorphism or cancer risk.

In our study, we examined common SNPs in *TP53* and *MDM2*, two genes central to cell cycle progression, cell survival, and genomic stability, and found no association with the well-studied exon 4 nonsynonymous SNP in *TP53*. This finding is consistent with null findings for this polymorphism in a Swedish study of 202 glioma and 164 meningioma cases (5), and a multicenter study of brain tumors (7), as well as one smaller study of brain tumors (4). Although a possible association with this polymorphism was reported in a previous study with adult and pediatric astrocytomas, DNA for that analysis was extracted from tumor samples, raising the possibility that the mutations occurred after tumor initiation (6). The potential importance of the *TP53* gene in brain tumor risk, however, has been shown by the fact that other polymorphisms have been associated with differences in risk: an intron 6 variant (rs1625895) in *TP53* has been associated with decreased risk of glioma and glioblastoma, as has the 1-2-2 haplotype of *TP53* (promoter-codon72-intron; ref. 7), whereas the CC-CG-CC haplotype (promoter-exon4-intron6) of *TP53* has been associated with increased risk of glioma and meningioma (5).

We observed decreased risk of glioma in individuals with the Ex12+162A>G polymorphism of the *MDM2* gene. The relationship between this *MDM2* polymorphism and brain tumor has not been previously reported in the literature. However, another common polymorphism in *MDM2* (IVS+309T>G, rs2279744) has been associated with increased risk of gastric cancer (26), esophageal cancer (27), lung cancer (28, 29), and bladder cancer (30) and may accelerate the risk of tumor formation in patients with familial breast cancer (31), Li-Fraumeni syndrome (32, 33), colorectal cancer (34), and gastric cancer (34). Given that MDM2 is a strong negative regulator of the p-53 cascade, one might expect a gene-gene interaction between polymorphisms in these two genes. Although previous studies have reported gene-gene interactions between *TP53* and the *MDM2* 309T>G polymorphism (34, 35), we did not detect any gene-gene interactions between the *MDM2* Ex12+162A>G and the *TP53* P72R polymorphisms.

This study had adequate statistical power to detect moderate to strong (but not weak) main effects of common genetic polymorphisms. Genotyping for the study was standardized; QC samples indicated high reproducibility of the genotyping results; and controls were in Hardy-Weinberg equilibrium for all polymorphisms under study.

Despite these strengths, we underscore the need for replication of our findings given the large number of false positives generated in genetic association studies (36), and the fact that our results did not meet the false discovery rate of 15%. Moreover, it is possible that the notable SNPs are actually in linkage disequilibrium with other causally relevant polymorphisms, but further work is needed to determine this. A more systematic approach toward coverage of these genes is warranted in follow-up studies. Although nonparticipation in the blood draw was higher among controls than cases, we believe that this is unlikely to be related to genotype, and thus unlikely to bias our results.

Our findings suggest that common variants in the apoptosis and cell cycle pathways may be important in brain tumor etiology. In particular, if our observations are verified, our results indicate that *CASP8, CCND1, CCNH*, and *MDM2* may be promising candidates for brain tumor susceptibility genes. Future research in this area should include more detailed coverage of SNPs within the genes implicated in this paper and examine related genes (e.g., caspases) in the apoptosis and cell cycle control pathways.

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Polymorphisms in Apoptosis and Cell Cycle Control Genes and Risk of Brain Tumors in Adults

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