Functional Polymorphisms in Folate Metabolism Genes Influence the Risk of Meningioma and Glioma

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Abstract

Folate metabolism plays an important role in carcinogenesis. To test the hypothesis that polymorphic variation in the folate metabolism genes *5,10-methylenetetrahydrofolate reductase* (*MTHFR*), *methionine synthase* (*MTRR*), and *methionine synthase reductase* (*MTR*) influences the risk of primary brain tumors, we genotyped 1,005 glioma cases, 631 meningioma cases, and 1,101 controls for the *MTHFR* C677A and A1298C, *MTRR* A66G, and *MTR* A2756G variants. *MTHFR* C677T-A1298C diplotypes were associated with risk of meningioma (P = 0.002) and glioma (P = 0.02); risks were increased with genotypes associated with reduced *MTHFR* activity. The highest risk of meningioma was associated with heterozygosity for both *MTHFR* variants [odds ratio (OR), 2.11; 95% confidence interval (95% CI), 1.42-3.12]. The corresponding OR for glioma was 1.23 (95% CI, 0.91-1.66). A significant association between risk of meningioma and homozygosity for *MTRR* 66G was also observed (OR, 1.41; 95% CI, 1.02-1.94). Our findings provide support for the role of folate metabolism in the development of primary brain tumors. In particular, genotypes associated with increased 5,10-methylenetetrahydrofolate levels are associated with elevated risk. (Cancer Epidemiol Biomarkers Prev 2008; 17(5):1195–202)

Introduction

Primary tumors of the central nervous system are the third most common tumor in men and sixth most common tumor in women between ages 35 and 49 years (1). Meningiomas and gliomas are the principal primary brain tumors (PBT) in adults (2), although the two tumor types are essentially biologically distinct.

Evidence for an inherited predisposition to glioma and meningioma is convincingly provided by several rare genetic syndromes [glioma: Li-Fraumeni syndrome (MIM151623), neurofibromatosis (MIM162200 and MIM101000), tuberous sclerosis (MIM191100), and Turcot's syndrome (MIM 276300); meningioma: neurofibromatosis type 2 (MIM101000) and Werner (MIM 277700) and Gorlin (MIM 109400) syndromes; ref. 3]. These syndromes do not, however, account for 2- to 3-fold elevated risk of glioma and meningioma in the relatives of patients with the same form of PBT (4), and it is likely that part of the inherited genetic risk is a consequence of low-risk variants, some of which may be common and hence detectable through association analyses.

Folate metabolism plays an important role in carcinogenesis due to its involvement in DNA methylation and nucleotide synthesis (5). Central to folate metabolism are the enzymes 5,10-methylenetetrahydrofolate reductase (MTHFR), methionine synthase (MTR), and methionine synthase reductase (MTRR), which play important and interrelated roles in folate metabolism (Fig. 1). The MTHFR enzyme occupies a pivotal position, balancing the homeostasis between DNA synthesis and methylation by catalyzing the irreversible conversion of

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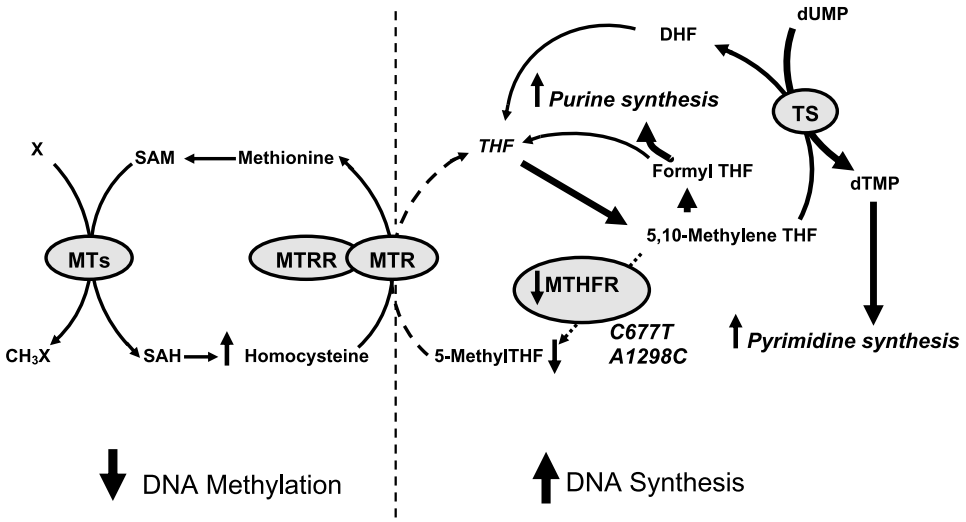

Figure 1. Schematic representation of folate metabolism and the possible effects of reduced MTHFR reductase activity on DNA synthesis and methylation. *DHF,* dihydrofolate; *MTs,* methyltransferases; *THF,* tetrahydrofolate; *SAM, S*-adenosylmethionine; *SAH, S*-adenosylhomocysteine; *TS,* thymidylate synthase.

5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate. The MTHFR substrate, 5,10-methylenetetrahydrofolate, is used by thymidylate synthase in the methylation of dUMP to dTMP, which is the sole *de novo* source of thymidine required for DNA synthesis and repair. The MTHFR product, 5-methyltetrahydrofolate, is the methyl group donor for the remethylation of homocysteine to methionine catalyzed by MTR in a reaction dependent on vitamin B12 as an intermediate methyl carrier. MTR may become inactive due to oxidation of its vitamin B12 cofactor, and restoration of MTR activity is dependent on reductive remethylation of vitamin B12 by MTRR. The genetic variants in the genes coding for MTHFR, MTR, and MTRR have in some cases been shown to affect directly on the function of the expressed proteins.

To examine whether variation in the genes participating in folate metabolism influence the risk of developing meningioma or glioma, we genotyped DNA from five case-control studies of PBT for *MTHFR* C677T and A1298C, *MTRR* A2756G, and *MTR* A66G.

Materials and Methods

Study Subjects. The study was based on five case-control studies of PBT that contributed to the Interphone Study (6) and that have been used previously for some candidate gene analyses (7). Briefly, the Interphone Study was an international multicenter case-control study coordinated by the IARC conducted between September 2000 and February 2004. The five study populations were the Thames regions of Southeast England; the Northern United Kingdom, including central Scotland, the West Midlands, West Yorkshire, and the Trent area; the Stockholm, Lund, Göteborg, and Umeå regions of Sweden; throughout Denmark; and in all regions of Finland, except Northern Lapland and Åland.

Adult PBT cases were identified through neurosurgery, neuropathology, oncology, and neurology centers and cancer registries. Eligible cases in the present study were patients with glioma, including glioblastoma [*International Classification of Diseases* (*ICD*), *Tenth Revision* code C71; *ICD for Oncology Second Edition* codes 9380-9384, 9390-9411, 9420-9451, and 9505] and meningioma [*ICD Tenth Revision* code C70; *ICD for Oncology Second Edition* codes 9530-9539], ages 18 to 69 years at diagnosis. Population-based controls were ascertained through general practitioner lists in the United Kingdom and randomly selected through the population registry in Nordic countries. Individuals were of the same age and residence criteria as cases and had no history of a cerebral tumor. Samples and clinicopathologic information from participants were obtained with informed consent and ethical review board approval in accordance with the tenets of the Declaration of Helsinki.

All cases of White European ethnicity for each country and with sufficient DNA quantity and quality were included in the genetic association studies. Controls were frequency matched on sex for each study center and disease using a random number generator. The number, sex, and age of cases and controls in each of the five studies analyzed in the current study were as follows: (*a*) glioma analysis: UK-North, 370 cases (230 males, 140 females; mean ± SD age at diagnosis, 49 ± 12 years) and 369 controls (231 males, 138 females; mean ± SD age, 51 ± 11 years); UK-Southeast, 211 cases (140 males, 71 females; mean ± SD age at diagnosis, 42 ± 11 years) and 214 controls (142 males, 72 females; mean ± SD age, 47 ± 9 years); Sweden, 197 cases (121 males, 76 females; mean ± SD age at diagnosis, 50 ± 13 years) and 197 controls (121 males, 76 females; mean ± SD age, 52 ± 12 years); Denmark, 128 cases (71 males, 57 females; mean ± SD age at diagnosis, 48 ± 12 years) and 131 controls (74 males, 57 females; mean ± SD age, 51 ± 12 years); Finland, 99 cases (56 males, 43 females; mean ± SD age at diagnosis, 48 ± 12 years) and 100 controls (37 males, 63 females; mean ± SD age, 53 ± 12 years). (*b*) meningioma analysis: UK-North, 174 cases (37 males, 137 females; mean ± SD age at diagnosis, 52 ± 10 years) and 175 controls (41 males, 134 females; mean ± SD age at recruitment, 50 ± 11 years); UK-Southeast, 121 cases (27 males, 94 females; mean ± SD age at diagnosis, 47 ± 8 years) and 123 controls (27 males, 96 females; mean ± SD age at recruitment, 46 ± 10 years); Sweden, 149 cases (50 males, 99 females; mean ± SD age at diagnosis, 55 ±

Table 1. Genotype counts, minor allele frequencies, and Hardy-Weinberg equilibrium *P* values for folate metabolism-related polymorphisms in meningioma and glioma cases and controls in the five series

Gene and SNP	Study center	Meningioma								Glioma							
		Cases			Controls			MAF	HWE	Cases			Controls			MAF	HWE
MTHFR																	
A1298C		AA	AC	CC	AA	AC	CC			AA	AC	CC	AA	AC	CC		
(rs1801131)	UK-North	80	73	20	94	64	17	0.28	0.26	174	162	33	203	130	36	0.27	0.04
	UK-Southeast	54	59	8	62	48	13	0.30	0.40	98	94	19	109	85	20	0.29	0.62
	Sweden	61	77	11	64	66	19	0.35	0.72	88	86	23	91	82	23	0.33	0.52
	Denmark	44	57	9	53	43	17	0.34	0.10	36	51	12	45	45	10	0.33	1.00
	Finland	38	31	8	37	32	8	0.31	0.79	54	64	10	64	54	13	0.31	0.84
C677T		CC	CT	TT	CC	CT	TT			CC	CT	TT	CC	CT	TT		
(rs1801131)	UK-North	57	98	19	73	78	24	0.36	0.74	168	160	41	155	168	46	0.40	1.00
	UK-Southeast	50	57	14	48	60	15	0.37	0.70	84	99	27	87	105	21	0.38	0.23
	Sweden	64	68	17	82	57	10	0.26	1.00	110	70	17	103	77	17	0.31	0.60
	Denmark	45	55	10	56	45	12	0.31	0.51	64	34	1	61	34	5	0.23	1.00
	Finland	46	26	5	47	25	5	0.23	0.52	65	51	12	60	57	14	0.36	1.00
MTRR																	
A66G		AA	AG	GG	AA	AG	GG			AA	AG	GG	AA	AG	GG		
(rs1801394)	UK-North	54	83	37	74	78	23	0.35	0.74	115	177	78	128	179	62	0.49	1.00
	UK-Southeast	41	57	23	39	59	25	0.44	0.85	69	97	45	66	101	47	0.58	0.49
	Sweden	39	84	26	53	74	22	0.40	0.73	68	94	35	66	97	34	0.51	1.00
	Denmark	41	47	22	40	55	18	0.40	1.00	42	50	7	40	37	23	0.54	0.02
	Finland	26	37	14	30	33	14	0.40	0.35	39	69	20	43	70	18	0.47	0.28
MTR																	
A2756G		AA	AG	GG	AA	AG	GG			AA	AG	GG	AA	AG	GG		
(rs1805087)	UK-North	113	54	7	106	60	8	0.22	1.00	256	101	13	240	115	13	0.19	1.00
	UK-Southeast	77	39	5	75	42	6	0.22	1.00	131	74	6	129	75	10	0.22	1.00
	Sweden	98	45	6	94	51	4	0.20	0.44	122	67	8	133	57	7	0.18	0.81
	Denmark	73	33	4	70	40	3	0.20	0.56	67	29	3	70	25	5	0.18	0.17
	Finland	50	24	3	56	17	4	0.16	0.10	74	49	5	82	45	4	0.20	0.59

Abbreviations: MAF, minor allele frequency in controls; HWE, Hardy-Weinberg equilibrium exact test *P* value.

9 years) and 149 controls (51 males, 98 females; mean ± SD age at recruitment, 52 ± 12 years); Denmark, 110 cases (35 males, 75 females; mean ± SD age at diagnosis, 52 ± 11 years) and 113 controls (35 males, 78 females; mean ± SD age at recruitment, 50 ± 11 years); Finland, 77 cases (14 males, 63 females; mean ± SD age at diagnosis, 52 ± 10 years) and 77 controls (14 males, 63 females; mean ± SD age at recruitment, 53 ± 11 years).

Single Nucleotide Polymorphism Genotyping and Data Manipulation. DNA was extracted from samples using conventional methodologies and quantified using PicoGreen (Invitrogen). Genotyping was conducted using Illumina GoldenGate Arrays (Illumina). DNA samples with GenCall scores < 0.25 at any locus were considered ''no calls.'' Cases and controls were genotyped in the same batches. To ensure quality of genotyping, duplicate samples were included in each 96-well sample plate.

Statistical Methods. Statistical analyses were undertaken using R and STATA Software (Stata). Single nucleotide polymorphism (SNP) genotype frequencies

Table 2. Risks of meningioma associated with folate metabolism polymorphisms

Gene and SNP	Genotype	Cases (%)	Controls (%)	OR (95% CI)	P_{trend}
MTHFR					
A1298C (rs1801131)	AA	277 (44.0)	310 (48.7)	1.00 (reference)	0.59
	AC	297 (47.1)	253 (39.7)	1.32 (1.04-1.66)*	
	CC	56 (8.9)	74 (11.6)	0.85 (0.58-1.24)	
	AC + CC	353 (56.0)	327 (51.3)	1.21 (0.97-1.51)	
C677T (rs1801133)	CC	262 (41.5)	306 (48.0)	1.00 (reference)	0.08
	CT	304 (48.2)	265 (41.6)	1.35 (1.07-1.71)*	
	TT	65 (10.3)	66 (10.4)	1.16 (0.79-1.70)	
	CT + TT	369 (58.5)	331 (52.0)	1.31 (1.05-1.64)*	
MTRR					
A66G (rs1801394)	AA	201 (31.9)	236 (37.0)	1.00 (reference)	0.03
	AG	308 (48.8)	299 (46.9)	1.21 (0.94-1.55)	
	GG	122 (19.3)	102 (16.0)	1.41 (1.02-1.94)*	
	AG + GG	430 (68.1)	401 (63.0)	1.26 (1.00-1.59)*	
MTR					
A2756G (rs1805087)	AA	411 (65.1)	401 (63.1)	1.00 (reference)	0.52
	AG	195 (30.9)	210 (33.0)	0.91 (0.71-1.15)	
	GG	25 (4.0)	25 (3.9)	0.98 (0.55-1.73)	
	AG + GG	220 (34.9)	235 (36.9)	0.91 (0.73-1.15)	

*$P < 0.05$.

Table 3. Risks of glioma associated with folate metabolism polymorphisms

Histology	Gene and SNP	Genotype	Cases (%)	Controls (%)	OR (95% CI)	P_{trend}
All	*MTHFR*					
	A1298C (rs1801131)	AA	450 (44.8)	512 (50.7)	1.00 (reference)	0.06
		AC	457 (45.5)	396 (39.2)	1.32 (1.09-1.58)*	
		CC	97 (9.7)	102 (10.1)	1.08 (0.80-1.47)	
		AC + CC	554 (55.2)	498 (49.3)	1.27 (1.06-1.51)*	
	MTHFR					
	C677T (rs1801133)	CC	491 (49.0)	466 (46.1)	1.00 (reference)	0.26
		CT	414 (41.3)	441 (43.7)	0.89 (0.74-1.07)	
		TT	98 (9.8)	103 (10.2)	0.90 (0.66-1.22)	
		CT + TT	512 (51.0)	544 (53.9)	0.89 (0.75-1.06)	
	MTRR					
	A66G (rs1801394)	AA	333 (33.1)	343 (33.9)	1.00 (reference)	0.75
		AG	487 (48.5)	484 (47.9)	1.04 (0.85-1.26)	
		GG	185 (18.4)	184 (18.2)	1.04 (0.80-1.34)	
		AG + GG	672 (66.9)	668 (66.1)	1.04 (0.86-1.25)	
	MTR					
	A2756G (rs1805087)	AA	650 (64.7)	654 (64.8)	1.00 (reference)	0.91
		AG	320 (31.8)	317 (31.4)	1.02 (0.84-1.23)	
		GG	35 (3.5)	39 (3.9)	0.90 (0.57-1.44)	
		AG + GG	355 (35.3)	356 (35.2)	1.00 (0.84-1.21)	
Glioblastoma	*MTHFR*					
	A1298C (rs1801131)	AA	198 (44.4)	512 (50.7)	1.00 (reference)	0.19
		AC	209 (46.9)	396 (39.2)	1.37 (1.08-1.73)*	
		CC	39 (8.7)	102 (10.1)	0.99 (0.66-1.48)	
		AC + CC	248 (55.6)	498 (49.3)	1.29 (1.03-1.62)*	
	C677T (rs1801133)	CC	211 (47.4)	466 (46.1)	1.00 (reference)	0.99
		CT	184 (41.3)	441 (43.7)	0.93 (0.73-1.18)	
		TT	50 (11.2)	103 (10.2)	1.07 (0.74-1.57)	
		CT + TT	234 (52.6)	544 (53.9)	0.96 (0.76-1.20)	
	MTRR					
	A66G (rs1801394)	AA	149 (33.3)	343 (33.9)	1.00 (reference)	0.52
		AG	208 (46.5)	484 (47.9)	0.98 (0.76-1.27)	
		GG	90 (20.1)	184 (18.2)	1.13 (0.82-1.55)	
		AG + GG	298 (66.7)	668 (66.1)	1.02 (0.81-1.30)	
	MTR					
	A2756G (rs1805087)	AA	291 (65.1)	654 (64.8)	1.00 (reference)	0.60
		AG	145 (32.4)	317 (31.4)	1.03 (0.81-1.31)	
		GG	11 (2.5)	39 (3.9)	0.64 (0.32-1.26)	
		AG + GG	156 (34.9)	356 (35.2)	0.99 (0.78-1.25)	
Astrocytoma	*MTHFR*					
	A1298C (rs1801131)	AA	158 (48.0)	512 (50.7)	1.00 (reference)	0.56
		AC	139 (42.2)	396 (39.2)	1.14 (0.88-1.49)	
		CC	32 (9.7)	102 (10.1)	1.02 (0.66-1.58)	
		AC + CC	171 (52.0)	498 (49.3)	1.12 (0.87-1.44)	
	C677T (rs1801133)	CC	163 (49.5)	466 (46.1)	1.00 (reference)	0.20
		CT	138 (41.9)	441 (43.7)	0.88 (0.68-1.15)	
		TT	28 (8.5)	103 (10.2)	0.77 (0.49-1.22)	
		CT + TT	166 (50.5)	544 (53.9)	0.86 (0.67-1.11)	
	MTRR					
	A66G (rs1801394)	AA	112 (34.0)	343 (33.9)	1.00 (reference)	0.92
		AG	157 (47.7)	484 (47.9)	1.00 (0.76-1.32)	
		GG	60 (18.2)	184 (18.2)	0.98 (0.68-1.41)	
		AG + GG	217 (66.0)	668 (66.1)	1.00 (0.77-1.30)	
	MTR					
	A2756G (rs1805087)	AA	208 (63.2)	654 (64.8)	1.00 (reference)	0.46
		AG	104 (31.6)	317 (31.4)	1.03 (0.78-1.35)	
		GG	17 (5.2)	39 (3.9)	1.34 (0.74-2.42)	
		AG + GG	121 (36.8)	356 (35.2)	1.07 (0.82-1.38)	
Oligodendroglioma	*MTHFR*					
	A1298C (rs1801131)	AA	42 (39.6)	512 (50.7)	1.00 (reference)	0.11
		AC	54 (50.9)	396 (39.2)	1.71 (1.12-2.63)*	
		CC	10 (9.4)	102 (10.1)	1.22 (0.59-2.52)	
		AC + CC	64 (60.4)	498 (49.3)	1.61 (1.07-2.43)*	
	C677T (rs1801133)	CC	53 (50.0)	466 (46.1)	1.00 (reference)	0.35
		CT	42 (39.6)	441 (43.7)	0.77 (0.50-1.19)	
		TT	11 (10.4)	103 (10.2)	0.85 (0.43-1.69)	
		CT + TT	53 (50.0)	544 (53.9)	0.79 (0.53-1.18)	
	MTRR					
	A66G (rs1801394)	AA	30 (28.3)	343 (33.9)	1.00 (reference)	0.97
		AG	62 (58.5)	484 (47.9)	1.41 (0.89-2.24)	
		GG	14 (13.2)	184 (18.2)	0.87 (0.45-1.69)	
		AG + GG	76 (71.7)	668 (66.1)	1.27 (0.81-1.98)	
	MTR					
	A2756G (rs1805087)	AA	66 (62.3)	654 (64.8)	1.00 (reference)	0.92
		AG	38 (35.8)	317 (31.4)	1.12 (0.73-1.72)	
		GG	2 (1.9)	39 (3.9)	0.50 (0.12-2.14)	
		AG + GG	40 (37.7)	356 (35.2)	1.06 (0.70-1.60)	

*$P < 0.05$.

in controls were tested for departure from Hardy-Weinberg equilibrium using an exact test. For each SNP, we tested the null hypothesis of no association with meningioma or glioma using the Cochran-Armitage trend test calculated by logistic regression. As age and sex were not significantly associated with meningioma or glioma risk within the data set, we restricted adjustment to study center. We assumed an additive codominant model by fitting the number of rare alleles carried as an ordinal covariate. The risk of meningioma or glioma associated with each SNP was quantified by heterozygote, homozygote, and minor allele carrier odds ratios (OR) and their 95% confidence intervals (95% CI) adjusted by logistic regression for study center. Because the 1,298 and 677 alleles of *MTHFR* influence the enzymatic activity of the expressed protein, we calculated risks of each form of PBT associated with diplotypes by conditional logistic regression, adjusted for study center, using the common homozygote wild-type genotype at both loci as the reference group. Analyses, including all available controls and adjusted for age, sex, and study center, did not yield substantially different results from those done with frequency-matched controls (Supplementary Tables S1 and S2).

Results

Genotypes were successfully generated for 2,744 of the 2,755 samples submitted (99.6%). Genotypes were obtained for 633 of 639 meningioma cases (99.1%), 1,010 of 1,013 glioma cases (99.7%), and 1,101 of 1,103 controls (99.8%). Ten samples were excluded from analysis due to unclear identity, leaving 2,734 samples: 631 meningioma cases, 1,005 glioma cases, and 1,098 controls. Completeness of genotyping was ≥99.9% for each of the individual loci with no difference in call rates between cases and controls. The concordance in SNP genotypes obtained between duplicate samples was 99.99%.

The distributions of *MTHFR* A1298C genotypes among glioma cases in UK-North and *MTRR* A66G among controls in Denmark were different from those expected under Hardy-Weinberg equilibrium although not significantly after correcting for multiple testing (Table 1). There were no significant differences in genotype frequencies between each of control series and minor allele frequencies of SNPs were in close agreement with the published data for European Caucasians (dbSNP).

Risks of meningioma and glioma associated with heterozygous and homozygous variant genotypes, both individually and combined, were computed (Tables 2 and 3). An influence of the *MTRR* A66G genotype on risk of meningioma but not glioma was observed. Homozygosity for the *MTRR* GG genotype was associated with a significantly increased risk of meningioma (OR, 1.41; 95% CI, 1.02-1.94). Similarly, the AG genotype was also associated with an increased risk albeit nonsignificantly (OR, 1.21; 95% CI, 0.94-1.55; Table 2).

Additionally, the *MTHFR* A1298C and C677T genotypes significantly influenced the risk of PBT. Heterozygosity for *MTHFR* A1298C was associated with an increased risk of both meningioma (OR, 1.32; 95% CI, 1.04-1.66) and glioma (OR, 1.32; 95% CI, 1.09-1.58). *MTHFR* C677T was also associated with risk of meningioma (OR, 1.35; 95% CI, 1.07-1.71) but not glioma (OR, 0.89; 95% CI, 0.74-1.07).

Of the glioma cases, 447 had been diagnosed with glioblastoma (*ICD Tenth Edition* codes 9440-1), 329 with astrocytoma (*ICD Tenth Edition* codes 9400-30), 106 with oligodendroglioma (*ICD Tenth Edition* codes 9450-1), and 123 with other glioma subtypes. Given biological differences between these histologic forms of glioma, we analyzed the association between genotypes and risk of each subtype. This analysis provided evidence that *MTHFR* genotypes were primarily associated with risk of glioblastoma and oligodendroglioma rather than astrocytoma (Table 3).

We investigated the combined effects of *MTHFR* SNP genotypes on risk of PBT by calculating risks associated with individual diplotypes (Tables 4 and 5). A significant and consistent association was observed between *MTHFR* diplotype and risk of both meningioma and glioma, with an increased risk associated with genotypes leading to reduced activity of the expressed protein. The association between *MTHFR* diplotype and glioma risk was primarily a consequence of an association with glioblastoma and oligodendroglioma subtypes (Table 5).

Discussion

Our findings suggest that folate metabolism polymorphisms play a role in determining the risk of developing PBT. Specifically, we observed an association with the functional variants of *MTHFR* A1298C and C677T and with the *MTRR* polymorphism A66G. Our analysis

Table 4. Association between *MTHFR* C667T and A1298C diplotypes and risk of meningioma

MTHFR diplotype 677_1298	Cases (%)	Controls (%)	OR (95% CI)	P_{OR}	$P_{diplotype}$
CC_AA	62 (9.8)	108 (17.0)	Reference		0.0019
CC_AC	144 (22.9)	124 (19.5)	2.03 (1.37-3.01)	0.0004	
CC_CC	56 (8.9)	74 (11.6)	1.33 (0.83-2.12)	0.24	
CT_AA	150 (23.8)	137 (21.5)	1.93 (1.30-2.85)	0.0010	
CT_AC	153 (24.3)	128 (20.1)	2.11 (1.42-3.12)	0.0002	
TT_AA	65 (10.3)	65 (10.2)	1.76 (1.10-2.81)	0.018	
TT_AC	0	1 (0.16)			
CT_CC	0	0			
TT_CC	0	0			
CC_AA	62 (9.8)	108 (17.0)	Reference		0.0015
CT/TT_AA	215 (34.1)	202 (31.7)	1.88 (1.30-2.72)	0.0008	
CC_AC/CC	200 (31.7)	198 (31.1)	1.77 (1.22-2.56)	0.0025	
CT/TT_AC/CC	153 (24.3)	129 (20.3)	2.09 (1.41-3.10)	0.0002	

also provides support for the rationale of conducting analyses based on the stratification of diplotypes to avoid confounding and maximize the power of any given study to identify associations as proposed previously (8).

A major strength of our study design is that we have based our analysis on five independent case-control series, thereby providing data on a large sample set for a relatively rare tumor. Potential limitations include the fact that only a subset of subjects interviewed in the International Interphone Study was analyzed. We have, however, documented previously that there are no salient differences in the characteristics of those donating a blood sample from those who only responded to the study questionnaire (9). Population stratification is a concern in all association studies as a source of bias as the frequency of genotypes for many polymorphic variants, such as *MTHFR* A1298C and C6677T, differ markedly between ethnic groups. We have sought to further minimize this form of bias by excluding subjects with ethnicity other than that of the country of recruitment. Survivorship is a potential source of bias if a variant influences prognosis. This is unlikely to be of serious concern in the present study as all cases were ascertained soon after diagnosis.

Functional studies have established that both heterozygous and homozygous variant genotypes of *MTHFR* 677T and 1298C result in reductions in enzyme activity

Table 5. Association between *MTHFR* C667T and A1298C diplotypes and risk of glioma

Histology	*MTHFR* diplotype 677_1298	Cases (%)	Controls (%)	OR (95% CI)	P_{OR}	$P_{diplotype}$
All	CC_AA	133 (13.3)	167 (16.6)	Reference		0.02
	CC_AC	261 (26.0)	196 (19.4)	1.68 (1.25-2.25)	0.0006	
	CC_CC	96 (9.6)	102 (10.1)	1.18 (0.82-1.69)	0.36	
	CT_AA	219 (21.9)	242 (24.0)	1.14 (0.85-1.52)	0.40	
	CT_AC	195 (19.5)	199 (19.7)	1.23 (0.91-1.66)	0.18	
	TT_AA	97 (9.7)	102 (10.1)	1.19 (0.83-1.71)	0.34	
	TT_AC	1 (0.10)	1 (0.10)			
	CT_CC	0	0			
	TT_CC	0	0			
	CC_AA	133 (13.3)	167 (16.6)	Reference		0.02
	CT/TT_AA	316 (31.5)	344 (34.1)	1.15 (0.87-1.52)	0.31	
	CC_AC/CC	357 (35.6)	298 (29.5)	1.51 (1.14-1.98)	0.004	
	CT/TT_AC/CC	196 (19.6)	200 (19.8)	1.23 (0.91-1.66)	0.18	
Glioblastoma	CC_AA	52 (11.7)	167 (16.6)	Reference		0.01
	CC_AC	121 (27.1)	196 (19.4)	2.01 (1.37-2.96)	0.0004	
	CC_CC	38 (8.5)	102 (10.1)	1.21 (0.74-1.97)	0.44	
	CT_AA	97 (21.7)	242 (24.0)	1.31 (0.89-1.94)	0.18	
	CT_AC	87 (19.5)	199 (19.7)	1.43 (0.96-2.14)	0.08	
	TT_AA	50 (11.2)	102 (10.1)	1.57 (0.99-2.50)	0.05	
	TT_AC	1 (0.2)	1 (0.10)			
	CT_CC	0	0			
	TT_CC	0	0			
	CC_AA	52 (11.7)	167 (16.6)	Reference		0.02
	CT/TT_AA	159 (35.7)	344 (34.1)	1.39 (0.96-2.00)	0.08	
	CC_AC/CC	147 (33.0)	298 (29.5)	1.74 (1.20-2.51)	0.0032	
	CT/TT_AC/CC	88 (19.7)	200 (19.8)	1.44 (0.96-2.15)	0.08	
Astrocytoma	CC_AA	56 (17.0)	167 (16.6)	Reference		0.86
	CC_AC	75 (22.8)	196 (19.4)	1.15 (0.77-1.72)	0.50	
	CC_CC	32 (9.7)	102 (10.1)	0.94 (0.57-1.55)	0.80	
	CT_AA	74 (22.5)	242 (24.0)	0.90 (0.61-1.35)	0.62	
	CT_AC	64 (19.5)	199 (19.7)	0.96 (0.63-1.45)	0.83	
	TT_AA	28 (8.5)	102 (10.1)	0.80 (0.48-1.35)	0.41	
	TT_AC	0	1 (0.10)			
	CT_CC	0	0			
	TT_CC	0	0			
	CC_AA	56 (17.0)	167 (16.6)	Reference		0.63
	CT/TT_AA	107 (32.5)	344 (34.1)	0.87 (0.60-1.27)	0.49	
	CC_AC/CC	102 (31.0)	298 (29.5)	1.08 (0.74-1.57)	0.70	
	CT/TT_AC/CC	64 (19.5)	200 (19.8)	0.95 (0.63-1.44)	0.82	
Oligodendroglioma	CC_AA	11 (10.4)	167 (16.6)	Reference		0.13
	CC_AC	32 (30.2)	196 (19.4)	2.56 (1.24-5.26)	0.01	
	CC_CC	10 (9.4)	102 (10.1)	1.44 (0.59-3.54)	0.42	
	CT_AA	20 (18.9)	242 (24.0)	1.17 (0.54-2.51)	0.69	
	CT_AC	22 (20.8)	199 (19.7)	1.57 (0.73-3.34)	0.25	
	TT_AA	11 (10.4)	102 (10.1)	1.48 (0.62-3.55)	0.38	
	TT_AC	0	1 (0.10)			
	CT_CC	0	0			
	TT_CC	0	0			
	CC_AA	11 (10.4)	167 (16.6)	Reference		0.07
	CT/TT_AA	42 (39.6)	344 (34.1)	1.26 (0.62-2.58)	0.52	
	CC_AC/CC	31 (29.2)	298 (29.5)	2.16 (1.08-4.33)	0.03	
	CT/TT_AC/CC	22 (20.8)	200 (19.8)	1.56 (0.73-3.32)	0.25	

compared with wild-type (10-12). Although the functional effects of *MTRR* A66G have not been fully established, *in vitro* experiments suggest that variant MTRR enzyme restores MTR activity less efficiently than wild-type, and the *MTRR* A66G genotype has been shown to influence plasma homocysteine levels in humans (13, 14). Coupled with the observation that individuals with the GG genotype are at increased risk of neural tube defects (15), a condition known to be associated with low folate levels, these data provide circumstantial evidence that this *MTRR* variant is functional.

It has been shown that aberrant genomic DNA methylation is associated with the development of most tumors, and folate metabolism plays an important role in carcinogenesis in general due to its involvement in DNA methylation and nucleotide synthesis. Reduced *MTHFR* activity inhibits the 5-methyltetrahydrofolate pathway, which can lead to increased levels of the MTHFR substrate 5,10-methylenetetrahydrofolate (a substrate for thymidylate synthetase, which catalyzes the synthesis of an essential precursor of *de novo* DNA synthesis) and decreased levels of the MTHFR product 5-methyltetrahydrofolate (a methyl group donor for the remethylation of homocysteine to methionine), thereby shifting the folate metabolism pathway away from methionine synthesis toward DNA synthesis and repair. The results of our study are consistent with an increased risk in subjects with reduced conversion of homocysteine to methionine due to either reduced MTRR enzyme activity or reduced activity upstream at the MTHFR enzyme, which could result in aberrant promoter methylation. The biological basis of PBT development is unclear. The role of aberrant methylation has, however, been documented in both gliomas and meningiomas (16-19). Given that studies have shown that the *MTHFR* 677TT genotype can be associated with decreased global DNA methylation and promoter-specific methylation in tumors (20), it is entirely plausible that the variants we have studied will affect the risk of PBT.

Compared with other cancer types, the role of polymorphic variants of the folate metabolism genes as risk factors for PBT has received comparatively little attention, and to our knowledge, only two studies have evaluated previously the role of variation in this pathway in development of glioma and meningioma. One small study based on analysis of 74 PBT patients and 94 controls found a higher frequency of *MTHFR* 677T genotypes in patients albeit nonsignificantly (21). A second study of 328 patients with glioblastoma multiforme and 400 controls found that the *MTR* 2756G allele was significantly underrepresented among cases (22). Although not significant, in our study of 447 glioblastoma cases, there was some support for a relationship between homozygosity and decreased risk (OR, 0.64; 95% CI, 0.32-1.26). Differences in association between variants and risk of glioma subtypes invite speculation that these reflect differences in the biology of the tumor types; however, we acknowledge that our study has limited power to robustly make this assertion.

There is increasing evidence implicating exogenous hormone use with risk of meningioma (23-25), and an interaction between hormone replacement therapy and *MTHFR* genotypes has been suggested (26). This is intriguing as meningiomas express functional progesterone and estrogen receptors and warrants further investigation.

Many studies have shown that the effect of variants, such as MTHFR C677T, on tumor risk is modified by dietary intake. Unfortunately, this type of data was unavailable to us and could not be included in our current analysis. However, it invites speculation as to the role of exogenous folate and other micronutrients as risk factors for PBT, which warrant exploration in future studies.

There has been considerable difficulties in unambiguously identifying causative exposures for PBT other than exposure to ionizing radiation. Hence, genetic associations for other candidate pathways might prove extremely valuable via the functional links they reveal and either endorse current etiologic hypotheses or suggest new ones that merit testing via gene/environment-specific hypotheses.

Here, we have found evidence that variation in folate metabolism genes affects the risk of developing both meningioma and glioma. However, as with all association studies, it is highly desirable that our findings are validated through replication in other case-control series.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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The R suite can be found at http://www.r-project.org/, Online Mendelian Inheritance in Man at http://www.ncbi.nlm.nih.gov/sites/entrez, and dbSNP: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?CMD=search&DB=snp.

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