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CLINICAL STUDY – PATIENT STUDY

Cancer susceptibility variants and the risk of adult glioma in a US case–control study

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Abstract Malignant gliomas are the most common and deadly brain tumors. Although their etiology remains elusive, recent studies have narrowed the search for genetic loci that influence risk. We examined variants implicated in recent cancer genome-wide association studies (GWAS) for associations with glioma risk in a US case–control study. Cases were identified from neurosurgical and neuro-oncology clinics at major academic centers in the Southeastern US. Controls were identified from the community or were friends or other associates of cases. We examined a total of 191 susceptibility variants in genes identified in published cancer GWAS including glioma. A total of 639 glioma cases and 649 controls, all Caucasian, were included in analysis. Cases were enrolled a median of 1 month following diagnosis. Among glioma GWAS-identified variants, we detected associations in *CDKN2B*, *RTEL1*, *TERT* and *PHLDB1*, whereas we did not find overall associations for *CCDC26*. Results showed clear heterogeneity according to histologic subtypes of glioma, with *TERT* and *RTEL* variants a feature of astrocytic tumors and glioblastoma (GBM), *CCDC26* and *PHLDB1* variants a feature of astrocytic and oligodendroglial tumors, and *CDKN2B* variants most prominent in GBM. No examined variant in other cancer GWAS was found to be related to risk after adjustment for multiple comparisons. These results suggest that GWAS-identified SNPs in glioma mark different molecular etiologies in glioma. Stratification by broad histological subgroups may shed light on molecular

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mechanisms and assist in the discovery of novel loci in future studies of genetic susceptibility variants in glioma.

Keywords Genotype · Glioma · Susceptibility · Single nucleotide polymorphism

Introduction

Glioma is one of the least understood and most aggressive human tumors. Risk factors for glioma remain largely unknown. A role for heredity is suggested by the occurrence of familial clustering of the disease. Relatives of patients with glioma are at increased risk for these tumors. Moreover, there are well-recognized genetic syndromes associated with increased risk including the Turcot and Li-Fraumeni syndromes, neurofibromatosis type 1 and multiple enchondromatosis. Genetic susceptibility for glioma has also been suggested in numerous case–control studies [1]. The availability of commercial arrays that capture most common variation in the genome has made possible genome-wide association studies (GWAS) which have successfully identified several hundred common genetic variants associated with cancer [2], some linked to multiple cancer phenotypes. Two GWAS in glioma concordantly identified variants in *CDKN2B, RTEL1* and *TERT* [3, 4] although results for other loci were not replicated in the two studies.

The GWAS of Wrensch et al. [3] involved genotyping 275,895 autosomal variants among 692 adult high-grade glioma (GBM and anaplastic astrocytoma) cases drawn mainly from the San Francisco Adult Glioma Study (AGS) and 3,992 controls. For replication, they analyzed 13 SNPs with $P < 10^{-6}$, which included markers in the genes *CDKN2B*, *RTEL1* and *TERT*, using independent data from 176 high-grade glioma cases and 174 controls from the Mayo Clinic. Validation analyses confirmed the presence of risk loci for glioma in the region of *CDKN2B* and *RTEL1*.

The GWAS of Shete et al. [4] involved a meta-analysis of two genome-wide association studies among Caucasian adults enrolled from the Interphone Study [5] in the UK and from MD Anderson Cancer Center in the US totaling 1,878 cases and 3,670 controls. Validation was provided by 3 independent European case–control studies totaling 2,545 cases and 2,953 controls. In a panel of 550K tagging SNPs, these investigators identified risk loci for glioma at *TERT*, *CCDC26*, *CDKN2B*, *RTEL1* and *PHLDB1*, each with a combined *P*-value of 1.07×10^{-8} or less. A total of 34 SNPs showed evidence of association at $P < 10^{-5}$ in the discovery GWAS, including multiple loci not identified in the GWAS of Wrensch et al. [3].

While findings from the two GWAS point to a role for *CDKN2A-CDK4* pathway and telomere function in glioma occurrence, the importance of other identified loci remains unclear. Moreover, the impact of these variants across the spectrum of glioma, a heterogenous tumor encompassing glioblastoma (GBM), the most common and aggressive astrocytic tumor, anaplastic and lower grade astrocytomas, oligodendroglioma and mixed oligoastrocytomas, has not yet been examined. Finally, the possibility that variants identified in GWAS of other primary cancers might impact glioma risk has not been explored. To shed further light on genes conferring susceptibility to glioma, we examined the panel of SNPs implicated in recent cancer GWAS in a large series of cases and controls enrolled in the Southeastern Study of Glioma in Adults (*GlioSE*), a multicenter, clinic-based case–control study conducted at medical centers in the Southeastern US, a region with excess brain tumor-associated mortality [6].

Subjects and methods

Study population

The study population was comprised of participants in an ongoing, clinic-based case–control study in the United States. Cases were comprised of English-speaking persons aged 18 and older with a recent diagnosis of primary (nonrecurrent) glioma identified in neurosurgery and neuro-oncology clinics at several major medical and oncology centers in the Southeastern US including Vanderbilt University Medical Center in Nashville, Tennessee; Moffitt Cancer Center & Research Institute in Tampa, Florida; the University of Alabama at Birmingham; Emory University in Atlanta, Georgia and the Kentuckiana Cancer Institute in Louisville, Kentucky.

Controls were comprised of friends, in-laws and other associates of the cases and persons sampled from the same communities as the cases identified from white page listings. Controls reporting a personal history of brain tumor were excluded from analysis.

Cases were enrolled a median of 1 month following the diagnosis of glioma (interquartile range: 2 weeks–1.7 months). The majority of eligible glioma patients (87%) participated in the study and provided a DNA sample. Of those not included in the study, 7% refused (generally due to illness) and 6% died before they could be approached or a DNA sample could be collected after providing informed consent. Among community controls, 49.6% of confirmed eligible households yielded a study participant.

DNA samples were self-collected by oral rinse or the saliva method using Oragene kits (www.dnagenotek.com). Collected samples were sent by participants or study

personnel to the Core Genotyping Facility at Vanderbilt (during the pilot phase) or to the Tissue Core at Moffitt for DNA extraction and storage.

The study was approved by Investigational Review Committees at each participating center and all subjects enrolled in the study provided written informed consent.

SNP genotyping

Genotyping was completed using Illumina's GoldenGate technology (Illumina, San Diego, CA) at the Center for Genome Technology at the Hussman Institute for Human Genomics, University of Miami. We developed a custom panel of 1536 SNPs that included 210 SNPs in genes linked to cancer in GWAS or large consortial studies (see Appendix 1). For glioma, the list included 34 SNPs reported in the GWAS of Shete et al. with a combined *P*-value of <0.0l in the discovery GWA and replication studies [4]. We also included 6 top nonredundant hits in the GWAS of Wrensch et al. [3]. Putatively functional SNPs among implicated genes were also included in the panel. In addition, we included 130 SNPs identified in GWAS of other cancers published up to Summer 2009 (http://www.genome.gov/gwastudies/). A list of genes, chromosomal location, and position for the SNPs included in the analysis is available online. Quality control samples (water, CEPH DNA, as well as blinded and unblinded DNA samples) were included in genotyping assays. Laboratory staff was blinded to the case–control status of the sample.

Genotyping with acceptable completion rates was accomplished for 191 of 210 (91%) attempted SNPs. All but one of the 191 SNPs (*RTEL1* rs7261546) satisfied the Hardy–Weinberg Equilibrium among controls at a nominal *P*-value of 0.01. Concordance of genotype calls in 95 blinded duplicate pairs ranged from 94 to 100% (mean, 99.8%) and the genotyping success rate ranged from 86 to 100% (mean, 95.0%).

A total of 699 glioma cases and 704 controls were submitted for genotyping. We excluded 33 cases (4.7%) and 31 controls (4.4%) with low call rates (<75%), and a further 27 cases (3.9%) and 24 controls (3.4%) who reported 1 or 2 non-Caucasian parents, leaving 639 glioma cases and 649 controls (including 550 community and 99 'friend' controls) available for general analysis. Based on a review of diagnostic pathology reports, the case group was comprised of 354 (55%) GBMs (ICD-O code [7] 9440/3); 153 (24%) lower grade pure astrocytic tumors including 77 grade 3 anaplastic astrocytoma (ICD-O 9401/3) and 76 grade 1 or 2 astrocytomas (ICD-O 9384/1, 9421/1, 9400/3, 9424/3); 99 (16%) mixed oligodendroglial and astrocytic tumors (ICD-O 9382/3) or pure oligodendrogliomas (ICD-O 9450/3, 9451/3), and 33 rare glioma variants ($N = 16$) or glioma with unspecified histology ($N = 13$) or unspecified histology and grade, e.g. 'glioma, NOS' ($N = 4$). As eligibility in the case–control study required a recent (within 3 months) diagnosis of glioma, only primary GBM and de novo anaplastic astrocytoma (e.g. nonrecurrences) were represented in the case group for these diagnoses.

A total of 349 of 354 patients with GBM had known vital status a minimum of 3 months after the diagnosis of glioma. Among them, 221 died from the tumor a median of 10.9 months (range: 0.8–57.9 months) following diagnosis. The median duration of follow up among 128 surviving patients was 10.9 months (range: 3.1–44.6 months).

Statistical analysis

Unconditional multivariate logistic regression adjusting for age and gender was used to estimate odds ratios and 95% confidence intervals for individual SNPs assuming an additive model. A score test of linear trend was conducted for each SNP using a three-level ordinal variable corresponding to the number of minor alleles for the SNP (0, 1 or 2). Multinomial logistic regression was used to evaluate genotype associations according to glioma subtypes including GBM, other astrocytic tumors, and a combination of oligodendroglial and mixed oligoastroglial tumors in the 16 'top-hit' (e.g. those with smallest *P*-values) SNPs reported in published glioma GWAS. Cox proportional hazards regression was used to assess whether genotypes were associated with mortality rates among the GBM cases. All regression analyses were performed using SAS (SAS Institute, Inc., Cary). To control the rate of type 1 error in multiple tests for association among loci identified for other cancers, we adjusted *P*-values using the method of Benjamini and Hochberg [8].

Results

The median age at enrollment was 55 years (range: 19–89) in cases and 58 years (range: 19–89) in controls. Males comprised 62% of cases and 57% of controls. The majority of subjects resided in Tennessee (30%) or Florida (25%), whereas a smaller number resided in Alabama (14%), Kentucky (14%), Georgia (11%), or another state (6%).

Table 1 shows results for genes representing top hits in glioma GWAS for *TERT*, *CCDC26*, *CDKN2B*, *PHLDB1* and *RTEL1*. With the exception of SNPs in *CCDC26,* we observed significant associations in all of the implicated SNPs. For *RTEL1*, in addition to the GWAS-identified SNPs, we examined 3 coding region SNPs (not shown). A nonsynonymous SNP (N124S; rs3848668) located upstream of the GWAS variants was not significantly associated with glioma risk ($P_{trend} = 0.252$). In contrast,

Table 1 Risk in relation to variants in genes identified in glioma GWAS

Gene	rs number	MAF	Genotype	OR (95% CI)	P_{trend}
TERT	rs2736100	0.48	TT	Ref	
			GT	1.25 (0.97–1.61)	
			GG	1.96 (1.41–2.70)	
			Per allele	1.37 (1.18–1.61)	0.000
	rs2853676	0.29	GG	Ref	
			AG	1.06 (0.84–1.33)	
			AA	1.78 (1.19–2.66)	
			Per allele	1.22 (1.03–1.44)	0.023
CCDC26	rs10464870	0.23	TT	Ref	
			CT	1.00 (0.79–1.27)	
			CC	0.80 (0.49–1.31)	
			Per allele	0.95 (0.79–1.14)	0.569
	rs891835	0.24	TT	Ref	
			GT	1.03 (0.81–1.30)	
			GG	0.88 (0.56–1.39)	
			Per allele	0.98 (0.82–1.17)	0.826
	rs6470745	0.19	AA	Ref	
			AG	1.23 (0.96–1.57)	
			GG	0.95 (0.51–1.79)	
			Per allele	1.13 (0.92–1.39)	0.232
	rs16904140	0.21	GG	Ref	
			AG	1.13 (0.89–1.44)	
			AA	0.75 (0.44–1.27)	
			Per allele	1.00 (0.83–1.21)	0.964
	rs4295627	0.18	TT	Ref	
			GT	1.27 (1.00–1.63)	
			GG	1.05 (0.55–2.00)	
			Per allele	1.18 (0.96–1.45)	0.115
CDKN2A-B	rs1063192	0.45	TT	Ref	
			CT	1.20 (0.92–1.56)	
			CC	1.43 (1.04–1.97)	
			Per allele	1.20 (1.02–1.40)	0.028
	rs2157719	0.43	AA	Ref	
			AG	1.24 (0.95–1.61)	
			GG	1.29 (0.93–1.81)	
			Per allele	1.15 (0.97–1.35)	0.100
	rs1412829	0.43	TT	Ref	
			CT	1.20 (0.93–1.55)	
			CC	1.46 (1.05–2.03)	
			Per allele	1.21 (1.03–1.42)	0.022
	rs4977756	0.41	AA	Ref	
			AG	1.32 (1.02–1.71)	
			GG	1.29 (0.92–1.80)	
			Per allele	1.16 (0.98–1.37)	0.078
PHLDB1	rs498872	0.31	CC	Ref	
			CT	1.36 (1.07–1.72)	
			TT	1.51 (1.04–2.18)	
			Per allele	1.27 (1.07–1.50)	0.005
	rs17748	0.23	CC	Ref	
			CT	1.00 (0.79–1.27)	
			TT	1.45 (0.89–2.35)	
			Per allele	1.09 (0.91–1.31)	0.333
RTEL1	rs6010620	0.26	AA	Ref	
			AG	1.35 (1.08–1.72)	
			GG	2.04 (1.23–3.33)	
			Per allele	1.39 (1.15–1.67)	0.001
	rs2297440	0.25	TT	Ref	
			CT	1.37 (1.06–1.75)	
			CC	1.92 (1.09–3.45)	
			Per allele	1.37 (1.12–1.67)	0.002
	rs4809324	0.11	TT	Ref	
			CT	1.10 (0.83–1.45)	
			CC	2.56 (0.97–6.75)	
			Per allele	1.21 (0.95–1.54)	0.130

Odds ratios (OR) and 95% confidence intervals (CI) are adjusted for age and gender

one synonymous (D664D; rs6062302) and one nonsynonymous (Q1042H; rs3208008) SNP, in tight linkage disequilibrium with 2 of the GWAS SNPs (rs6010620 and rs2297440) had strong associations with glioma risk ($P_{trend} < 0.001$).

We examined 'top-hit' glioma GWAS SNPs according to grade and histological subtype of glioma (Table 2). *TERT* rs2736100 was significantly associated only with high-grade tumors (GBM) ($P_{trend} < 0.001$) whereas weaker and nonsignificant associations were observed for lower grade pure astrocytic and oligodendroglial tumors (a combination of pure oligodendroglial and mixed astrocytic). A similar pattern was observed for the *CDKN2B* SNPs. The results for *CCDC26*, null in the combined analyses, suggested a significant association for oligodendroglial tumors and a borderline association for lower grade pure astrocytic tumors. *PHLDB1* rs498872 was significant only in lower grade astrocytic tumors ($P_{trend} = 0.001$) with a borderline association also observed among the oligodendrogliomas ($P_{trend} = 0.042$). For the *RTEL1* SNPs, associations were confined to the astrocytic tumors whereas these SNPs had no association with oligodendroglial tumors.

A case-only analysis (Table 3) showed a deficit of variant alleles in *RTEL1* rs4809324 (OR: 0.50; 95% CI 0.28–0.91; $P_{trend} = 0.023$) in oligodendroglial tumors when compared to GBM; the same pattern was observed for all 4 *CDKN2B* SNPs. In contrast, an excess of variant alleles was observed in *CCDC26* rs4809324, rs16904140 and rs4295627 for oligodendroglial and astrocytic tumors

Table 2 Risk in relation to variants in glioma GWAS-identified genes according to histologic subtype and grade

Gene	rs number	GBM only (*N*=353 cases)		Lower grade astrocytic tumors (*N*=153 cases)		Oligodendroglial tumors (*N*=99 cases)	
		OR (95% CI)	P_{trend}	OR (95% CI)	P_{trend}	OR (95% CI)	P_{trend}
TERT	rs2736100	1.52 (1.25–1.82)	0.000	1.28 (1.00–1.67)	0.053	1.10 (0.81–1.49)	0.529
	rs2853676	1.23 (1.00–1.50)	0.046	1.23 (0.92–1.64)	0.159	1.11 (0.77–1.60)	0.562
CCDC26	rs10464870	0.85 (0.68–1.07)	0.167	0.98 (0.73–1.33)	0.898	1.18 (0.82–1.68)	0.379
	rs891835	0.85 (0.68–1.06)	0.145	1.06 (0.80–1.42)	0.665	1.31 (0.93–1.85)	0.118
	rs6470745	0.85 (0.66–1.10)	0.219	1.43 (1.05–1.95)	0.025	1.79 (1.22–2.63)	0.003
	rs16904140	0.80 (0.63–1.01)	0.061	1.23 (0.92–1.65)	0.165	1.48 (1.05–2.10)	0.026
	rs4295627	0.90 (0.70–1.17)	0.429	1.52 (1.11–2.09)	0.010	1.83 (1.25–2.67)	0.002
CDKN2B	rs1063192	1.34 (1.11–1.62)	0.003	1.20 (0.92–1.56)	0.188	0.94 (0.68–1.31)	0.712
	rs2157719	1.28 (1.05–1.56)	0.013	1.15 (0.88–1.52)	0.308	0.90 (0.64–1.26)	0.524
	rs1412829	1.31 (1.08–1.59)	0.006	1.29 (0.99–1.69)	0.064	0.95 (0.67–1.34)	0.762
	rs4977756	1.29 (1.05–1.57)	0.013	1.17 (0.89–1.54)	0.248	0.93 (0.67–1.31)	0.695
PHLDB1	rs498872	1.13 (0.93–1.38)	0.230	1.55 (1.18–2.02)	0.001	1.40 (1.01–1.95)	0.042
	rs17748	0.95 (0.76–1.19)	0.678	1.26 (0.94–1.69)	0.127	1.29 (0.89–1.86)	0.174
RTEL1	rs6010620	1.35 (1.09–1.69)	0.008	1.61 (1.18–2.27)	0.004	1.22 (0.83–1.79)	0.313
	rs2297440	1.35 (1.05–1.72)	0.016	1.54 (1.10–2.17)	0.013	1.23 (0.83–1.82)	0.302
	rs4809324	1.45 (1.09–1.94)	0.011	1.01 (0.67–1.53)	0.944	0.78 (0.45–1.38)	0.400

Odds ratio (OR) and 95% confidence interval (CI) in dominant model adjusted for age and gender

Table 3 Case-only analysis of GWAS-identified SNPs according to histologic subtype of glioma

Gene	rs number	Astrocytic tumors versus GBM (referent)		Oligodendroglial tumors versus GBM (referent)		Oligodendroglial versus astrocytic tumors (referent)	
		OR (95% CI)	P_{trend}	OR (95% CI)	P_{trend}	OR (95% CI)	P_{trend}
TERT	rs2736100	0.85 (0.63–1.15)	0.294	0.70 (0.50–1.00)	0.048	0.83 (0.58–1.19)	0.303
	rs2853676	0.91 (0.67–1.25)	0.573	0.79 (0.55–1.15)	0.226	0.87 (0.59–1.28)	0.480
CCDC26	rs10464870	1.03 (0.72–1.48)	0.872	1.19 (0.78–1.83)	0.324	1.19 (0.78–1.183)	0.419
	rs891835	1.21 (0.86–1.71)	0.267	1.45 (0.99–2.13)	0.060	1.19 (0.80–1.77)	0.386
	rs6470745	1.53 (1.04–2.25)	0.032	1.84 (1.19–2.84)	0.007	1.20 (0.77–1.87)	0.414
	rs16904140	1.48 (1.03–2.14)	0.035	1.83 (1.21–2.77)	0.004	1.23 (0.81–1.87)	0.324
	rs4295627	1.58 (1.07–2.32)	0.021	1.87 (1.21–2.89)	0.005	1.19 (0.76–1.85)	0.451
CDKN2B	rs1063192	0.82 (0.60–1.11)	0.197	0.63 (0.44–0.91)	0.013	0.78 (0.53–1.13)	0.181
	rs2157719	0.84 (0.61–1.15)	0.280	0.64 (0.44–0.94)	0.021	0.77 (0.52–1.13)	0.180
	rs1412829	0.92 (0.68–1.25)	0.598	0.68 (0.47–0.98)	0.037	0.74 (0.50–1.08)	0.115
	rs4977756	0.86 (0.62–1.18)	0.351	0.67 (0.46–0.98)	0.038	0.78 (0.53–1.16)	0.218
PHLDB1	rs498872	1.40 (1.03–1.91)	0.031	1.22 (0.85–1.76)	0.276	0.87 (0.60–1.27)	0.471
	rs17748	1.39 (1.00–1.93)	0.053	1.33 (0.90–1.95)	0.156	0.96 (0.64–1.42)	0.823
RTEL1	rs6010620	1.22 (0.83–1.79)	0.314	0.86 (0.56–1.31)	0.473	0.70 (0.45–1.10)	0.124
	rs2297440	1.18 (0.78–1.77)	0.437	0.88 (0.57–1.38)	0.587	0.75 (0.47–1.19)	0.228
	rs4809324	0.70 (0.44–1.10)	0.119	0.51 (0.28–0.92)	0.024	0.73 (0.39–1.35)	0.314

Odds ratios (OR) and 95% confidence intervals (CI) adjusted for age and gender

when compared to GBM. Deleterious alleles in *PHLDB1* rs498872 were approximately 40% more common for astrocytic tumors than GBM (OR: 1.41; 95% CI 1.04–1.92; $P_{trend} = 0.028$). Genotype frequencies were not significantly different comparing astrocytic and oligodendroglial tumors.

In the GWAS of Shete et al. of 34 SNPs with a high level of significance ($P < 10^{-5}$) in the GWA studies, 20 did not achieve an accepted threshold for genome-wide significance ($P < 5 \times 10^{-7}$) after pooling with the replication studies. We examined all of these SNPs (not shown) and observed nominally significant associations in 2 of

them. The latter included an intergenic SNP (rs7300686) on chromosome 12 (overall $P_{trend} = 0.012$) in which the association was significant only among astrocytic tumors (per allele OR: 1.39; $P_{trend} = 0.012$) and an intergenic SNP (rs1384847) on chromosome 4 (overall $P_{trend} = 0.043$) in which the association was driven mainly by association with GBMs (per allele OR: 1.46; $P_{trend} = 0.028$). All of the remaining 18 variants were null in the present data (not shown).

We included in our panel 3 SNPs identified uniquely in the GWAS of Wrensch et al. [3] which included primarily GBMs: *CSF1R* rs10079250 on chromosome 5, rs11163687 on chromosome 7 near the gene *TTLL7* and rs11823971 on chromosome 11 near the gene *PDE2A*. There was no evidence of LD between the SNP on chromosome 5 and the *TERT* SNPs ($R^2 = 0.008$, $D' = 0.37$ for rs2736100 in *TERT* in HapMap) or the SNP on chromosome 11 and the *PHLDB1* SNP ($R^2 = 0.008$ and $D' = 0.20$ in HapMap). We did not find evidence of association for these 3 SNPs with glioma risk, overall, or for GBM only, although all 3 variants were uncommon (MAF: 5, 13 and 9% in controls, respectively), limiting our power. However, we did observe borderline associations within astrocytic tumors for rs11163687 (OR: 0.58; 95% CI 0.36–0.93; $P_{trend} = 0.024$) and rs10079250 (OR: 1.88; 95% CI: 1.09–3.25; $P_{trend} = 0.023$).

Finally, we examined associations of 'top-hit' glioma GWAS SNPs with survival among the GBM cases. None of the GWAS-identified SNPs in Table 1 were found to be related to patient survival times with statistical significance.

Among 116 examined SNPs identified in GWAS of other primary cancers (Supplemental data), none were significantly associated with glioma risk after adjustment for multiple testing.

Discussion

The current study provides evidence that susceptibility variants identified in published glioma GWAS differ among GBM, lower grade astrocytic tumors and oligodendrogliomas and mixed tumors. While these categories are broad, they define tumors with different clinical behavior and/or arising from cells with distinct functions in the CNS [9] making distinguishing patterns in the genomic background plausible. Results have implications for interpreting published GWAS and for planning future GWAS which these data suggest should consider potential heterogeneity according to recognized histopathological subtypes of glioma.

In the present study, variants in *CCDC26* and *PHLDB1* were associated primarily with astrocytic and oligodendroglial tumors. *CCDC26* rs4295627 provided the strongest signal in the Shete GWAS [4]. Consistent with the GWAS of Wrensch et al. [3] we did not detect association for *CCDC26* rs4295627 or 4 other variants in *CCDC26* also linked to risk in the Shete GWAS in a combined analysis. However, we were able to detect strong associations among astrocytic tumors and tumors with an oligodendroglial component. This finding helps reconcile results of the 2 GWAS as the study by Wrensch et al. was limited to high-grade astrocytic tumors (84% GBM and 16% anaplastic astrocytomas). The SNP rs498872 which maps to the 5′UTR of *PHLDB1* provided the fifth-strongest signal in the Shete GWAS [4]. This variant was also not detected by Wrensch et al. and in the current study, an association was limited to lower grade astrocytomas and oligodendrogliomas. Taken together, the findings suggest that both *CCDC26* and *PHLDB1* play a limited role in the genesis of primary GBM.

In these data, variants in *TERT*, *RTEL* and *CDKN2B* were primarily a feature of astrocytic tumors and GBM. *TERT* rs2736100, *CDKN2B* rs4977756 and *RTEL1* rs6010620 provided the second, third and fourth strongest signal, respectively, in the GWAS of Shete et al. [4] and were concordantly identified in the GWAS of Wrensch et al. [3] (although the *TERT* SNP was detected only in the discovery (AGS) data in the latter GWAS). In the current study, *TERT* rs2736100 was significantly associated only with GBM whereas *RTEL1* rs6010620 was more strongly associated with the lower grade astrocytic tumors. Variants in *CDKN2B* were most prominent in GBM although odds ratios were nonsignificantly elevated also for astrocytic tumors. The suggestion from these data that genetic variation in *TERT*, *RTEL* and *CDKN2B* play a more prominent role in tumors with astrocyte lineage should be explored in larger studies.

We examined association of the variants in these genes (listed in Table 1) with mortality rates in our series of GBM patients and could demonstrate no significant associations. A recent study [10] suggested that *CCDC26* rs10464870 and rs891835 and *RTEL1* rs2297440 and rs6010620 predict long-term survival (≥36 months) in GBM. We failed to detect overall association of these variants with GBM mortality. Too few deaths occurred more than 36 months after diagnosis (a total of 5 deaths among 9 patients which survived that duration) for a meaningful analysis according to survival time.

In addition to top-hit variants in these 5 genes, we detected associations in 2 intergenic SNPs identified the Shete GWAS (rs1384847 and rs7300686) [4]. Both of these SNPs were significant in the discovery GWA but not the replication studies. Neither variant was in linkage disequilibrium with any implicated SNP on chromosomes 4 and 12, respectively, in the Wrensch GWAS (not shown)

[3]. Furthermore, there was no evidence in our data that associations involving these variants were confined to lower grade astrocytic tumors and/or oligodendrogliomas. Three variants identified uniquely in the Wrensch GWAS [3] were not associated with risk in current study, overall, or among high-grade tumors. Further research is needed to establish the relevance of these SNPs in gliomagenesis.

We examined SNPs identified in GWAS of other cancers published up to Summer 2009 and found modest associations in several variants, although none remained significant after adjustment for multiple comparisons. An exception is *TERT* rs2736100 which was identified in both glioma GWAS and has also been implicated in GWAS of testicular germ cell tumors [11] and lung cancer [12–14]. An intronic SNP (rs402710) located in *CLPTM1L* near *TERT*, identified in lung cancer GWAS [14], was marginally associated with risk in the current data ($P_{\text{trend}} = 0.039$) before adjustment; this SNP is uncorrelated with *TERT* rs2736100 and *TERT* rs2853676 (not shown). The *CLPTM1L* SNP was marginally associated with GBM mortality in the current data (HR: 1.40; 95% CI 1.07–1.84; $P_{trend} = 0.015$) whereas the *TERT* SNPs had no association with mortality. The SNP rs6983267 near *POU5F1P1* on chromosome 8 is multicancer susceptibility marker linked to prostate [15], colon [16], and a range of other cancers [17]. This variant showed no association with glioma in the present series. Two breast cancer variants in *FGFR2* (rs1219648 and rs2981582) [18] were each marginally associated with glioma risk before adjustment; that amplification of *FGFR2* has been noted in GBM [19] suggests these variants may have a causal role in glioma and should be examined in larger studies.

Strengths of the current study include the relatively large sample size for a study of a rare tumor such as glioma, pathologic confirmation of all cases, and the limited potential influence of survival bias given exceptionally rapid enrollment of cases. However, the study had several limitations. Our study had limited power to detect associations with rare variants and those with modest relative risks, and the study size only permitted us to examine associations according to broad histological strata of glioma. Our classification of tumors by histology was based on the diagnostic pathology reports and some misclassification was possible [20]. We note, however, that our results are consistent with those in a recent report that showed distinct patterns of association for the five most prominent SNPs in the Shete GWAS according to WHO grade of glioma [21]. As in the current study, risk alleles for *TERT* rs2736100 were strongly correlated with the diagnosis of GBM whereas carrier frequencies of risk alleles of *CCDC26* rs4295627 and *PHLDB1* rs498872 were inversely correlated with GBM histology (*RTEL1* rs6010620 had a stronger association with GBM in that study). In both series, *CDKN2B* rs4977756 risk allele frequency did not vary strongly by histology in astrocyte lineage tumors. Concordance of results in the two studies suggests that classification in the current study was reasonably accurate at least among the astrocytic tumors. Finally, we could not evaluate effects of genotypes by race as ~98% of subjects enrolled in the study were Caucasian. Racial admixture among Caucasians in the study was not evaluated (approximately 93% of cases and 95% of controls reported European ancestry in one or both parents) and may have diluted some associations.

Rapid advances have recently been made in understanding genes and signaling networks involved in the incidence and pathogenesis of glioma [22]. The current results suggest that stratification by broad histological subgroups has the potential to uncover novel risk loci and may shed additional light on genetic susceptibility to glioma in future studies.

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Conflict of interest The authors have no conflicts of interest.

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