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Genetic variants in telomerase-related genes are associated with an older age at diagnosis in glioma patients: evidence for distinct pathways of gliomagenesis

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Background. Genome-wide association studies have implicated single nucleotide polymorphisms (SNPs) in 7 genes as glioma risk factors, including 2 (*TERT, RTEL1*) involved in telomerase structure/function. We examined associations of these 7 established glioma risk loci with age at diagnosis among patients with glioma.

Methods. SNP genotype data were available for 2286 Caucasian glioma patients from the University of California, San Francisco ($n = 1434$) and the Mayo Clinic ($n = 852$). Regression analyses were performed to test for associations between "number of risk alleles" and "age at diagnosis," adjusted for sex and study site and stratified by tumor grade/histology where appropriate.

Results. Four SNPs were significantly associated with age at diagnosis. Carrying a greater number of risk alleles at rs55705857 (*CCDC26*) and at rs498872 (*PHLDB1*) was associated with younger age at diagnosis ($P = 1.4 \times 10^{-22}$ and $P = 9.5 \times 10^{-7}$, respectively). These SNPs are stronger risk factors for oligodendroglial tumors, which tend to occur in younger patients, and their association with age at diagnosis varied across tumor subtypes. In contrast, carrying more risk alleles at rs2736100 (*TERT*) and at rs6010620 (*RTEL1*) was associated with older age at diagnosis ($P = 6.2 \times 10^{-4}$ and $P = 2.5 \times 10^{-4}$, respectively). These SNPs are risk factors for all glioma grades/histologies, and their association with age at diagnosis was consistent across tumor subgroups.

Conclusions. Carrying a greater number of risk alleles might be expected to decrease age at diagnosis. However, glioma susceptibility conferred by variation in telomerase-related genes did not follow this pattern. This supports the hypothesis that telomerase-related mechanisms of telomere maintenance are more associated with gliomas that develop later in life than those utilizing telomerase-independent mechanisms (ie, alternative lengthening of telomeres).

Keywords: age at diagnosis, glioma, single nucleotide polymorphism, telomerase, telomere.

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Glioma, the most common central nervous system cancer in adults, generally has poor prognosis. Glioblastoma, the most common and most aggressive form of glioma, has a median patient survival time of just 15 months from diagnosis under current standard of care.[1] While prognosis is better for low-grade astrocytic tumors and tumors with an oligodendroglial component, over time these tumors all progress to high-grade glioma.[2]

Gliomagenesis is a complex and multifaceted process influenced by both inherited and acquired genetic variation. Glioma risk loci in 7 genes have been confirmed in genome-wide association studies.[3–6] Several of these risk loci are found in genes previously implicated in gliomagenesis due to their mutation in glioma-associated hereditary cancer syndromes (*TP53, CDKN2B/ANRIL*) or their alteration in glioma tumors (*TP53, CDKN2B/ANRIL, EGFR*).[7–9] Risk loci in 2 genes involved in telomerase structure and function (*TERT* [telomerase reverse transcriptase] and *RTEL1* [regulator of telomere elongation helicase 1]) had not been implicated in gliomagenesis prior to genome-wide association studies, but telomerase activation has been observed in ~90% of all human cancers.[10]

Telomeres act as a protective cap at the end of chromosomes but are progressively shortened during mitotic divisions.[11] Telomere depletion ultimately leads to replicative senescence, limiting the proliferative capacity of cells. With activation of telomerase, an enzyme that adds DNA sequence repeats to telomeres, dividing cells can replace lost telomeric DNA and continue proliferating.[10] *TERT* is a key component of human telomerase, and *RTEL1* is needed to allow telomerase-dependent telomere extension to proceed effectively.[12,13] Of the tumors that do not maintain telomere length through activation of telomerase, a significant subset activates a secondary pathway: alternative lengthening of telomeres (ALT).[14]

Inheriting an increased number of glioma risk alleles might be expected to decrease age at diagnosis among glioma patients by reducing the number of somatic mutations an individual must acquire to initiate tumor formation or by facilitating the accumulation of such mutations. To investigate this, we examined the associations of known glioma risk loci with age at diagnosis in glioma patients from the University of California, San Francisco (UCSF) and the Mayo Clinic. Caucasian glioma patients were genotyped at single nucleotide polymorphisms (SNPs) in 7 genes associated with glioma risk in previous genome-wide association studies, including variants in *TERT, EGFR, CCDC26, CDKN2B/ANRIL, PHLDB1, TP53,* and *RTEL1*. Associations were also calculated within histologic subgroups, stratified by tumor *IDH*-mutation status, and pooled across study sites. Interactions between age and risk SNPs were also examined in case-control comparisons.

Materials and Methods

Study Population

This study included European-ancestry glioma patients and controls from UCSF (1434 cases, 1114 controls) and the Mayo Clinic (852 cases, 789 controls). Both participating institutions received institutional review board approval, and informed consent was obtained from subjects. Patient recruitment methods have been described in detail elsewhere.[3,15] Pathology review was performed as previously described.[3,16]

SNP Selection

SNPs in 7 different genes have been significantly associated with glioma risk in previous genome-wide case-control studies.[3–6] We chose the SNP most strongly associated with glioma risk in previous case-control analyses for all association testing described in this paper.[15,17] Thus, we analyzed only 1 glioma risk SNP from each region.

Genotyping

Genotyped on GoldenGate custom genotyping arrays (Illumina) were 810 UCSF cases, all Mayo cases, 512 UCSF controls, and all Mayo controls. GoldenGate genotyping was performed by the UCSF Genome Center and Mayo Genotyping core facilities as previously described.[15] Samples were submitted in 96-well plates containing intra- and interplate replicates to ensure genotype reproducibility.

Genotypes for an additional 606 UCSF cases and 602 controls were extracted from an Illumina 370k genome-wide SNP chip genotyped as part of a previous study.[3] The 370k platform does not contain a probe for rs55705857 (*CCDC26*) or rs78378222 (*TP53*). Therefore, genotype data were available for only 810 total UCSF cases and 512 UCSF controls at rs55705857. However, 461 additional UCSF cases were directly genotyped at rs78378222 (*TP53*) as previously described,[6] for a total of 1271 UCSF cases and 512 UCSF controls with genotype data at this locus.

For both study sites, samples with genotyping array call rates $<95\%$ were excluded from analysis. SNPs with genotyping call rates $<95\%$ in any site were excluded from all analyses. To exclude poorly genotyped SNPs, any SNP with a Hardy-Weinberg equilibrium P-value $<.001$ in controls, stratified by site, was removed from further analyses.

Statistical Analysis of SNP Associations

For the case-only analyses, the correlation between number of risk alleles and age at diagnosis was assessed using Pearson's correlation coefficient calculated in SAS v9.1.3, stratified by study site. Regression analyses were conducted using linear regression in PLINK v1.07 (http://pngu.mgh.harvard.edu/purcell/plink/), adjusted for sex and study site, assuming an allelic additive model in which the regression coefficient represents the effect of each extra copy of the risk allele.[18] Because at 3 loci the glioma risk allele is not the minor allele (rs2736100, rs11979158, rs6010620), the reference allele was forced using the "—reference-allele" command so that regression coefficients corresponded to the change in age at diagnosis

associated with each additional copy of the glioma risk allele, as defined by previous case-control studies. Linear regression coefficients were also calculated in analyses stratified by tumor histology, and heterogeneity across histologic strata was assessed using Cochran's Q and I^2. For all linear regression models, residual plots, including normal probability plots, were examined for departures from normality, excess skew, and kurtosis. All reported *P*-values are 2-sided. For the primary study results listed in Table 2, a total of 11 statistical comparisons were considered (1 comparison per SNP for the 3 loci displaying no heterogeneity of effect across tumor types; 2 comparisons per SNP for the 4 loci displaying significant heterogeneity of effect across tumor types). A strict Bonferroni correction for these 11 comparisons corresponds to an adjusted significance threshold of 4.5×10^{-3} (0.05/11).

Case-control association statistics for rs55705857 (*CCDC26*), rs498872 (*PHLDB1*), rs2736100 (*TERT*), and rs6010620 (*RTEL1*) were calculated using logistic regression in PLINK, adjusting for sex and study site. In order to assess variation in SNP effect size at different patient ages, glioma cases and controls were divided into 5 age strata, in years: <40, 40–49, 50–59, 60–69, and 70+. Reported case-control associations are for an allelic additive model, where odds ratios are for each additional copy of the known risk allele.

Assessment of IDH-*Mutation Status*

UCSF tumor specimens were sequenced to identify *IDH1* and *IDH2* mutations using previously described methods.[19] The region spanning the R132 codon of *IDH1* and the region spanning the R172 codon of *IDH2* were amplified by PCR with M13-tagged primers to facilitate amplification and sequencing. Products were run on a 1.5% agarose gel and subsequently sequenced in both directions at the UCSF Genomics Core Facility according to the manufacturer's protocol. Sequences were analyzed with Applied Biosystems Sequence Scanner Software v1.0. Mayo tumor specimens were assayed for *IDH1* mutations using pyrosequencing and for *IDH2* mutations using both pyrosequencing and Sanger sequencing as previously described.[20]

Results

A total of 2286 glioma patients (1434 UCSF, 852 Mayo) and 1903 controls (1114 UCSF, 789 Mayo) had acceptable genotyping call rates and were included in our analyses. Subject characteristics, including histopathologic classification of glioma cases, are outlined in Table 1. The 7 SNPs reported in this study passed all call-rate and Hardy-Weinberg equilibrium thresholds.

Cases were stratified into 2 groups: purely astrocytic tumors (glioblastoma, anaplastic astrocytoma, grade 2 astrocytoma) and tumors with an oligodendroglial component (oligodendroglioma and mixed oligoastrocytoma). Four SNPs were strongly and significantly associated with age at diagnosis in one or both subgroups (Table 2), and these results were consistent in UCSF and Mayo patients (Table S1). The *CCDC26* risk allele was associated with a 7.25-year decreased age at diagnosis in the astrocytic subgroup (95% confidence interval [CI] = 5.44–9.07 yr; $P = 1.2 \times 10^{-14}$) and a 2.81-year decreased age at diagnosis in the oligodendroglial tumor subgroup (95% CI = 0.96–4.65 yr; $P = 3.1 \times 10^{-3}$) (Table 2). Rs55705857 in *CCDC26* is known to be a risk factor primarily for oligodendroglial and *IDH*-mutated gliomas,[15] but a significant association with decreased age at diagnosis was observed in all grade and histology subgroups (Table S2), and also when analysis was restricted to *IDH*-mutant tumors (Table S3). Because the magnitude of this effect appeared stronger in the astrocytic tumor subgroups than in the oligodendroglial subgroups (Table S2), analyses are presented stratified by tumor cell type.

Carrying a greater number of rs498872 risk alleles in *PHLDB1* was associated with a 2.30-year younger age at diagnosis in the combined regression analysis of all astrocytic tumors (95% CI = 1.35–3.25 yr; $P = 2.1 \times 10^{-6}$). There was statistically significant heterogeneity in this effect between the purely astrocytic tumors and tumors with an oligodendroglial component (P_{HET} =

Table 1. Demographic and tumor histology characteristics of the UCSF Adult Glioma Study and the Mayo Clinic glioma patients and controls used in the genetic association analyses

	UCSF Samples			Mayo Samples			Combined		
	n	Mean Age, yr (SD)	% Male	*n*	Mean Age, yr (SD)	% Male	*n*	Mean Age, yr (SD)	% Male
Glioblastoma	887	56.6 (12.1)	64.7	330	55.7 (12.4)	62.7	1219	56.4 (12.2)	64.2
Anaplastic astrocytoma	170	46.9 (15.0)	55.9	188	48.4 (14.8)	54.8	358	47.7 (14.9)	55.3
Grade II astrocytoma	115	42.5 (13.5)	66.1	70	42.1 (13.6)	55.7	185	42.4 (13.5)	62.2
Mixed oligoastrocytoma	64	39.0 (11.3)	59.4	166	39.0 (11.5)	54.8	230	39.0 (11.4)	56.1
Oligodendroglioma	179	45.0 (12.1)	54.7	98	41.6 (10.7)	51.0	277	43.8 (11.7)	53.4
All histologies	1434[a]	52.0 (14.0)	62.3	852	48.1 (14.4)	57.5	2288	50.5 (14.3)	60.5
Controls	1114	56.9 (15.2)	54.4	789	49.8 (14.1)	57.0	1903	53.9 (15.2)	55.5

[a]Numbers by histologic type add to 1434 because 12 astrocytomas were of indeterminate grade and 7 gliomas had no histology information.

3.5×10^{-3}), where no significant association was observed ($P = .73$).

While risk alleles in *CCDC26* and *PHLDB1* were associated with a reduced age at diagnosis, risk alleles in the telomerase-related genes *TERT* and *RTEL1* were associated with an increased age at diagnosis across all tumor grades and histologies (Table 2 and Table S2). Among all glioma patients, controlling for sex and study site, each additional copy of the rs2736100 risk allele in *TERT* was associated with a 1.47-year increased age at diagnosis (95% CI = 0.63–2.32 yr; $P = 6.2 \times 10^{-4}$). This association was consistent in the astrocytic and oligodendroglial tumor subgroups ($I^2 = 0.0$).

Carrying a greater number of risk alleles at the other telomerase-related SNP, rs6010620 in *RTEL1*, was associated with a 2.02-year increased age at diagnosis in analysis of all gliomas (95% CI = 0.94–3.10 yr; $P = 2.5 \times 10^{-4}$). Like the *TERT* association, this association did not display heterogeneity across grades or histologies ($I^2 = 0.0$) (Table 2 and Table S2).

Because *TERT* and *RTEL1* function within a common pathway, the associations of rs2736100 and rs6010620 with age at diagnosis were also modeled jointly to assess whether the observed associations were independent or possibly synergistic in nature. Including both SNPs in a regression model did not attenuate associations, indicating that both rs2736100 and rs6010620 are independently associated with age at diagnosis. Inclusion of an interaction term in the model (rs2736100 genotype*rs6010620 genotype) did not reveal the presence of any significant effect modification ($P = .78$). Combining the risk allele dosage into a single ordinal variable representing the total number of risk SNPs in a telomerase-related gene (range, 0–4) supported this conclusion, as each additional risk SNP was associated with a 1.72-year older age at diagnosis (95% CI = 1.06–2.37 yr; $P = 2.5 \times 10^{-7}$) (Table 3).

In order to determine whether the significant effects observed in the case-only analyses of *CCDC26*, *PHLDB1*, *TERT*, and *RTEL1* were similar in case-control analyses, case-control SNP associations were calculated in 5 different age strata. Both rs55705857 (*CCDC26*) and rs498872 (*PHLDB1*) conferred the greatest risk for glioma in the youngest age group and the least risk for glioma in the oldest age group, consistent with the case-only associations previously discussed (Fig. 1A and B). Conversely, rs2736100 (*TERT*) conferred the greatest risk for glioma in the oldest age group (odds ratio [OR] = 1.68, 95% CI = 1.30–2.17) and the least risk for glioma in the youngest age group (OR = 1.19, 95% CI = 0.98–1.45) (Fig. 1C). Additionally, rs6010620 (*RTEL1*) conferred a greater risk for glioma in people aged ≥70 years compared with its effect in those <40 years (OR = 1.62, 95% CI = 1.07–2.26 vs OR = 1.08, 95% CI = 0.85–1.37, respectively) (Fig. 1D). The ORs observed for SNPs in *TERT* and *RTEL1*, which grow in magnitude with subject age, are consistent with the case-only associations previously discussed.

Among patients with oligodendroglial tumors, an increased number of risk alleles at rs1412829 in

Table 2. Association between age at diagnosis and number of risk alleles at known glioma risk loci in combined UCSF Adult Glioma Study and Mayo Clinic patients, stratified by tumor cell type

SNP	Chromosome	Gene	Risk Allele	All Gliomas		Purely Astrocytic Tumors[a]		Tumors With an Oligo Component[b]		Heterogeneity Tests[c]	
				Effect (SE)[d]	*P*[e]	Effect (SE)[d]	*P*[e]	Effect (SE)[d]	*P*[e]	*P*	I^2
Rs2736100	5	*TERT*	C	1.47 (0.43)	**6.2×10^{-4}**	1.38 (0.48)	**4.3×10^{-3}**	0.85 (0.74)	0.25	0.55	0
Rs11979158	7	*EGFR*	A	0.48 (0.60)	0.43	0.78 (0.68)	0.25	−1.37 (1.05)	0.19	0.086	66.16
Rs55705857	8	*CCDC26*	G	-	-	−7.25 (0.93)	**1.2×10^{-14}**	−2.81 (0.94)	**3.1×10^{-3}**	**8.0×10^{-4}**	91.15
Rs1412829	9	*CDKN2B/ANRIL*	G	-	-	0.55 (0.47)	0.24	−1.91 (0.77)	0.013	**6.2×10^{-3}**	86.66
Rs498872	11	*PHLDB1*	A	-	-	−2.30 (0.48)	**2.1×10^{-6}**	0.25 (0.73)	0.73	**3.5×10^{-3}**	88.31
Rs78378222	17	*TP53*	C	-	-	−2.49 (1.35)	0.065	4.25 (2.45)	0.084	**0.016**	82.77
Rs6010620	20	*RTEL1*	G	2.02 (0.55)	**2.5×10^{-4}**	1.80 (0.63)	**4.3×10^{-3}**	0.81 (0.91)	0.37	0.37	0

[a]Includes glioblastomas ($n = 1217$), and grades II–III astrocytomas ($n = 555$).
[b]Includes oligodendrogliomas ($n = 277$) and mixed oligoastrocytomas ($n = 230$).
[c]*P*-values from Cochran's Q-statistic, testing for heterogeneity in beta across strata of tumor cell type (purely astrocytic vs oligodendroglial). I^2 can range from 0 to 100, where larger numbers indicate a greater level of heterogeneity across histology strata.
[d]Effect size (in y) is generated from a linear regression model where age at diagnosis is the dependent variable and number of risk alleles is the independent variable, controlling for sex and study site. Positive values indicate older age at diagnosis with an increasing number of risk alleles. Negative values indicate younger age at diagnosis with an increasing number of risk alleles.
[e]*P*-values are 2-sided and are derived from the regression model (H_0: beta = 0). A total of 11 statistical comparisons are considered (1 comparison per SNP for the 3 loci displaying no heterogeneity of effect across tumor types; 2 comparisons per SNP for the 4 loci displaying significant heterogeneity of effect across tumor types). A strict Bonferroni correction for these 11 comparisons corresponds to an adjusted significance threshold of 4.5×10^{-3} (0.05/11). *P*-values in bold were considered statistically significant.

Table 3. Association between age at diagnosis and number of risk alleles in a telomerase-related gene (*TERT* or *RTEL1*) in combined UCSF Adult Glioma Study and Mayo Clinic glioma patients

	Number of Glioma Risk Alleles in a Telomerase-Related Gene					Effect (SE)	*P*
	0	1	2	3	4		
n	19	155	611	1010	491	1.72 (0.33)	2.5×10^{-7}
Average age at diagnosis, yr	41.7	47.1	49.4	50.9	52.5		

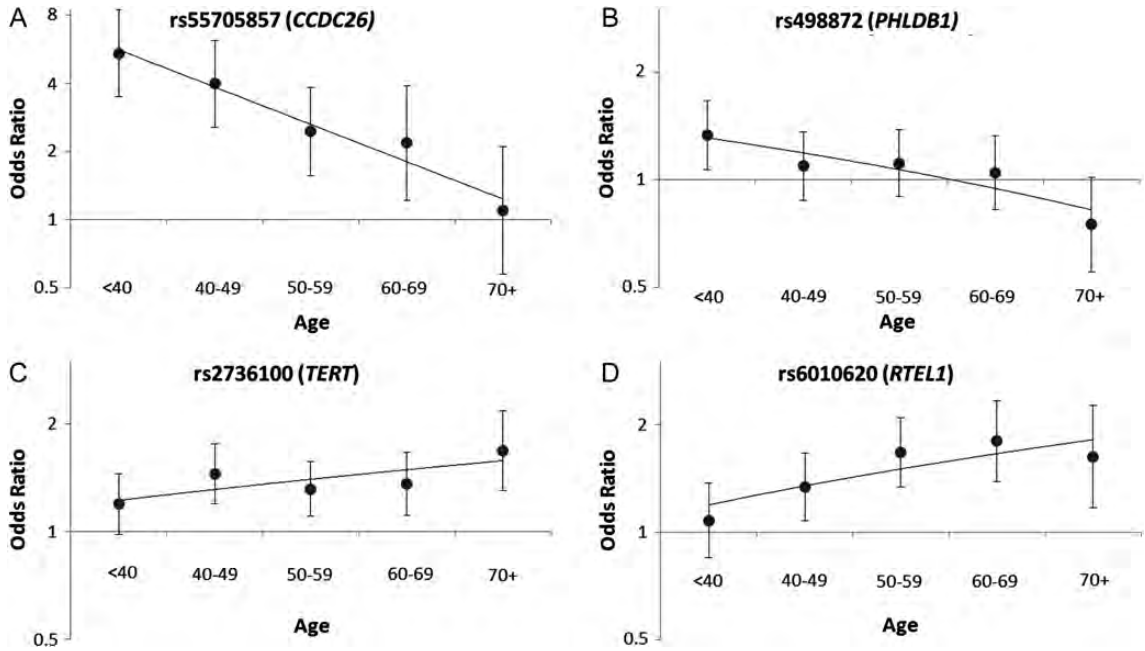

Fig. 1. Changes in the magnitude of glioma risk associated with 4 SNPs across subject age strata in case-control analyses. Odds ratios for glioma were calculated in case-control analyses adjusted for sex and study site; 95% CIs appear around each effect estimate. The *y* axis is represented on a log-scale (base 2), ranging from 0.50 to 8.0 for Fig. 1A and 0.50 to 2.0 for Fig. 1B–1D.

CDKN2B/ANRIL was associated with a 1.91-year younger age at diagnosis (95% CI = 0.41–3.42 yr; $P = .013$), but no association was observed in the astrocytic tumor group ($P_{HET} = 6.2 \times 10^{-3}$) (Table 2). Although we have previously shown that rs1412829 confers increased risk for *IDH* wild-type tumors but not *IDH*-mutant tumors,[17] the rs1412829 glioma risk allele was also associated with a decreased age at diagnosis among patients with *IDH*-mutant tumors (Table S3).

The effect of the glioma risk allele in *TP53* on age at diagnosis also appeared to differ across the astrocytic/oligodendroglial strata ($P_{HET} = 0.016$), but power to detect stratified associations at this locus is limited because the risk allele frequency is just 3.2% in cases (Table 2).

Discussion

SNP rs55705857 (*CCDC26*) is a risk factor for oligodendroglial tumors and also for *IDH*-mutated astrocytomas, but not *IDH* wild-type astrocytomas.[15] While this SNP is associated with a younger age at diagnosis in all glioma strata in our sample, the effect is significantly more pronounced in the purely astrocytic tumor group, consistent with the earlier age at diagnosis observed for *IDH*-mutated astrocytic tumors compared with *IDH* wild-type astrocytic tumors.[21] Similarly, rs498872 (*PHLDB1*) is associated with risk for *IDH*-mutated tumors but not with risk for *IDH* wild-type tumors.[22] In our analyses, this SNP was associated with a younger age at diagnosis only among astrocytic tumors. Adult oligodendroglial tumors typically develop earlier in life than adult astrocytic tumors. Therefore, the stronger effects of *CCDC26* and *PHLDB1* gene variants on age at diagnosis in patients with astrocytic tumors suggests that these tumors undergo gliomagenesis through a pathway similar to that of oligodendroglial tumors.

If there is a pathway of gliomagenesis shared by oligodendrogliomas and early-onset astrocytomas, *IDH1/2* mutation is the obvious candidate marker of such a pathway. Mutations in *IDH1/2*, *CIC*, and *FUBP1* all correlate with an oligodendroglial tumor histology, younger age at diagnosis, and better survival. Together, mutations in *IDH1/2* and *ATRX* correlate with lower-grade

astrocytomas and secondary glioblastomas, which have a younger age at onset than primary glioblastoma.[23] Tumors with *ATRX* mutations nearly universally activate the ALT pathway, immortalizing cells through a telomerase-independent mechanism.[23,24] ALT has been observed in 7%–15% of adult glioblastomas and oligodendrogliomas[23–25] and in ~75% of grades II and III astrocytomas and mixed oligoastrocytomas.[23,26] Additionally, the ALT phenotype is associated with a younger age at diagnosis among patients with *IDH*-mutant glioma, irrespective of tumor grade.[23] Our data support the hypothesis that tumors with an earlier age at diagnosis are more likely to maintain their telomeres through a telomerase-independent mechanism (eg, ALT) than are tumors with a later age at diagnosis.

Risk alleles in *TERT* and *RTEL1*, genes related to telomerase structure and function, were associated with a later age at diagnosis in glioma patients. These SNPs confer an increased risk for glioma, independent of tumor grade or histology.[16,17] As a result, it may not be surprising that the effect of these SNPs on age at diagnosis is also consistent across grade, histology, and *IDH*-mutation strata. Specific variants in *TERT* have previously been associated with longer telomere length and increased lifespan in humans.[27,28] The ability to maintain or lengthen telomeres confers resistance to replicative senescence and supports proliferative potential.[10] A cell with increased telomere length, or increased telomerase activity, may have greater capacity to circumvent replicative senescence, a major mechanism of tumor suppression. Variants in *RTEL1* and *TERT* may also place cells in a preactivated state, making them more liable to initiate telomerase activity following normal growth arrest and thereby achieving a critical step in cancer progression. Such a potential pathway would correspond with a tumor that develops later in life through acquired mutations and an inherent predisposition to eschew growth arrest.

It is worth noting that rs2736100 (*TERT*) and rs6010620 (*RTEL1*) have risk allele frequencies greater than 50% in our case sample. Although rs2736100 confers increased risk for lung cancer, testicular germ cell cancer, and glioma,[29] the risk variant has an allele frequency of 52.7% in Caucasian HapMap samples. Rs6010620 in *RTEL1* has an even higher risk allele frequency of 75.7% in Caucasian HapMap samples.[30] Although the neoplastic diseases associated with these variants are for the most part postreproductive, and therefore the risk alleles are less susceptible to selection, it is uncommon to have such high cancer risk allele frequencies. Considering that variants in telomerase-related genes may increase individual lifespans but may also increase cellular lifespans in a manner that resists tumor suppression, both positive and negative selective pressures may influence allele frequency at these loci.

Because several cancers are associated with *TERT* SNPs and nearly 90% of neoplasms activate telomerase, understanding the role of these genes in carcinogenesis is of paramount importance. Our data indicate that patients with a greater number of *TERT* and *RTEL1* risk SNPs develop glioma later in life. These results suggest that such tumors maintain telomeres through the canonical telomerase-based mechanism, distinct from the ALT mechanism associated with tumors appearing earlier in life. Because telomerase-based cancer therapeutics are currently undergoing clinical trials,[10] our results may also be useful in identifying which patients can benefit most from enrollment in these studies.

Supplementary Material

Supplementary material is available online at Neuro-Oncology (http://neuro-oncology.oxfordjournals.org/).

Conflict of interest statement. None declared.

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