Relationship between genetically determined telomere length and glioma risk

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Abstract

Background. Telomere maintenance is increasingly recognized as being fundamental to glioma oncogenesis with longer leukocyte telomere length (LTL) reported to increase risk of glioma. To gain further insight into the relationship between telomere genetics and risk of glioma, we conducted several complementary analyses, using genome-wide association studies data on LTL (78 592 individuals) and glioma (12 488 cases and 18 169 controls).

Methods. We performed both classical and summary Mendelian randomization (SMR), coupled with heterogeneity in dependent instruments tests, at genome-wide significant LTL loci to examine if an association was mediated by the same causal variant in glioma. To prioritize genes underscoring glioma-LTL associations, we analyzed gene expression and DNA methylation data.

Results. Genetically increased LTL was significantly associated with increased glioma risk, random-effects inverse variance weighted ORs per 1 SD unit increase in the putative risk factor (odds ratio [OR] SD) 4.79 (95% confidence interval: 2.11–10.85; \(P = 1.76 \times 10^{-4}\)). SMR confirmed the previously reported LTL associations at 3q26.2 (\(TERC\); \(P_{\text{SMR}} = 1.33 \times 10^{-5}\)), 5p15.33 (\(TERT\); \(P_{\text{SMR}} = 9.80 \times 10^{-27}\)), 10q24.33 (\(STN1\) alias \(OBFC1\); \(P_{\text{SMR}} = 4.31 \times 10^{-9}\)), and 20q13.3 (\(STMN3/RTEL1\); \(P_{\text{SMR}} = 2.47 \times 10^{-4}\)) glioma risk loci. Our analysis implicates variation at 1q42.12 (\(P_{\text{SMR}} = 1.55 \times 10^{-2}\)), 6p21.3 (\(P_{\text{SMR}} = 9.76 \times 10^{-3}\)), 6p22.2 (\(P_{\text{SMR}} = 5.45 \times 10^{-3}\)), 7q31.33 (\(P_{\text{SMR}} = 6.52 \times 10^{-3}\)), and 11q22.3 (\(P_{\text{SMR}} = 8.89 \times 10^{-4}\)) as risk factors for glioma risk. While complicated by patterns of linkage disequilibrium, genetic variation involving \(PARP1\), \(PRRC2A\), \(CARMIL1\), \(POT1\), and \(ATM-NPAT1\) was implicated in the etiology of glioma.

Conclusions. These observations extend the role of telomere-related genes in the development of glioma.

Key Points

- We explore the genetic relationship between telomere maintenance and glioma development.
- Our analyses implicate genetic variation at a number of novel loci as risk factors for glioma.
- Our observations extend the role of telomere-related genes in glioma development.

Gliomas account for approximately 80% of adult primary malignant brain tumors, and they are generally associated with poor clinical outcomes irrespective of treatment. The etiology of glioma is poorly understood, the only modifiable risk factor consistently linked to risk being exposure to ionizing radiation, which accounts for very few cases. Our understanding of inherited predisposition to glioma has been transformed by genome-wide association studies (GWAS), which have so far identified 25 loci influencing risk. Many of the risk loci exhibit subtype specificities, with a recent study proposing...
2q37.3 and 7p22.3 as additional risk loci for low-grade gliomas. Elucidating the functional basis of these risk loci is, therefore, an important step toward the development of testable hypotheses regarding the biological processes involved in the development of glioma.

Large-scale sequencing of glioma has highlighted dysregulated telomere biology as central to glioma oncogenesis, with somatic mutations in the promoter of the telomerase reverse transcriptase gene (TERT) being common in primary glioblastoma multiforme (GBM) and grades II and III oligodendroglioma. Telomeres protect chromosome ends during DNA replication, shortening with each normal cell division until a critical point when apoptosis ensues. The enzyme telomerase, which consists of TERT and telomerase RNA component (TERC), is involved in the maintenance of telomere length. It is generally essential for tumors to avoid senescence and apoptosis by acquisition of telomere length maintenance strategies, often through telomerase reactivation. In glioma, this is typically through upregulation of telomerase by TERTp mutation or the alternative lengthening of telomeres pathway by ATRX mutation.

Telomere length is determined by the balance of processes that shorten and lengthen the telomere resulting in variation between individuals at the same age. The relationship between telomere length and cancer risk has been widely investigated, and longer leukocyte telomere length (LTL) has been reported as a risk factor for a number of cancers, including glioma, implying a general relationship. Heritable factors contribute to the variation in telomere length and GWAS have identified multiple loci influencing LTL. With genetically predicted higher LTL reported to be associated with increased risk of glioma.

To gain further insight into the relationship between telomere genetics and risk of glioma, we conducted complementary analyses, making use of LTL GWAS data of 78,592 individuals and a GWAS of 12,488 glioma cases and 18,169 controls. Firstly, we conducted Mendelian randomization analyses to estimate the effect of LTL on glioma risk. Secondly, we performed summary Mendelian randomization (SMR) and heterogeneity in dependent instruments (HEIDI) tests at the 20 genome-wide significant LTL loci to examine if an association was mediated by the same causal variant (indicative of pleiotropy) (Figure 1). Thirdly, to prioritize genes that are most likely influenced by the genetic variation at each locus, we leveraged large-scale human genomic data integrated with tissue gene expression and DNA methylation data, coupled with knowledge-driven manual curation. Finally, to estimate the genome-wide heritability and genetic correlation between LTL and risk of glioma, we conducted a cross-trait linkage disequilibrium adjusted kinship (LDAK) analysis.

Materials and Methods

Ethics

Ethical approval for this study was not required as our analyses were based on summary statistics from published GWAS or the data were publicly accessible and no individual-level data were used.

Datasets

Glioma GWAS data were obtained from the most recent meta-analysis of 12,488 cases and 18,169 controls of European descent. Different subtypes of glioma, defined in part by malignancy grade (eg, pilocytic astrocytoma—World Health Organization [WHO] grade I, diffuse “low-grade” glioma—WHO grade II, anaplastic glioma—WHO grade III, and glioblastoma [GBM]—WHO grade IV) can be distinguished. For brevity, we considered gliomas as being either GBM (n = 6183) or non-GBM (n = 5820).

LTL GWAS data were obtained from the recent European Network for Genetic and Genomic Epidemiology (ENGAGE), European Prospective Investigation into Cancer (EPIC)-CVD, and EPIC-Interact consortium study involving 78,592 individuals of European descent.

We explored the functional basis of associations by analyzing gene expression and methylation in tissues. Specifically, expression quantitative trait loci (e-QTL) data from peripheral whole blood (CAGE, n = 2765) and brain tissue (Brain eMeta, n = 1194) were from Lloyd-Jones et al. and Qi et al., respectively. Brain methylation quantitative trait loci (me-QTL) data (n = 1160) were from Qi et al. Individual methylation probes were attributed to gene transcripts if the genomic location of the probe overlapped the start and end site of Ensembl v101 transcript locations lifted from hg38 to hg37 (for each gene, the
canonical transcript was used; otherwise, the longest transcript was used).33

$R^2$ and $D'$ metrics of linkage disequilibrium (LD) between single nucleotide polymorphisms (SNPs) in the European population (CEU) were obtained using LDlink.34 HaploReg v4.1 was used to investigate the presence of tissue-specific “Enhancer Histone Marks” in all variants in strong LD ($r^2 > 0.8$) with lead LTL-associated SNPs.35

Statistical Analysis

Two-sample MR was conducted using the TwoSampleMR R package.36 For each SNP, the chromosome position, the effect estimate expressed in standard deviations (SD) of the trait per-allele, and the corresponding standard error (SE) were recovered (Supplementary Table 1). SNPs were only considered as potential instruments if they were associated at $P < 5 \times 10^{-8}$ in a GWAS of European populations and had a minor allele frequency (MAF) > 0.01. To avoid co-linearity between SNPs, correlated SNPs were excluded (LD threshold, $r^2 ≥ 0.01$). For each SNP, causal effect estimates were generated for glioma, GBM, and non-GBM tumors as ORs per 1 SD unit increase in the putative risk factor ($\text{OR}_{\text{sub}}$), with 95% confidence intervals (CIs), using the Wald ratio. Causal effects were estimated using a random-effects inverse weighted variance (IVW-RE) model, which assumes that each SNP identifies a different causal effect. These causal effects are averaged to elucidate the true causal effect, due to balanced pleiotropy.36 To assess the robustness of our findings, we compared the causal estimates and associated P-values using weighted median and weighted mode-based methods (Supplementary Table 2).37,38 We examined the potential impact of outlying and pleiotropic SNPs on causal estimates using a leave-one-out strategy (Supplementary Table 3). The directional pleiotropy was assessed using MR-Egger regression (Supplementary Table 2).39 The heterogeneity ($P$) within the SNP associations between LTL and each glioma dataset (all glioma, GBM, and non-GBM) was calculated from Cochran’s Q-value (Supplementary Table 4).40,41

We carried out SMR analyses, as per Zhu et al.42 To distinguish pleiotropic associations from those driven by linkage, we tested for HEIDI in each region; under the hypothesis of pleiotropy, $P_{\text{SMR}}$ values for SNPs in LD with the causal variant should be identical. Default settings were used adopting a window +/- 1 Mb from the SMR probe’s location. To account for multiple testing, a Benjamini-Hochberg false discovery rate (FDR) correction (FDR $q ≤ 0.05$) was applied. The HEIDI test result was based upon the 20 maximally associated SNPs within a region, after pruning for LD, and a $P$-value < 0.01 was considered significant. For the SMR analysis of e-QTL and me-QTL data, we limited our analysis to LTL-glioma concordant associations signals.13

The heritability of LTL and glioma and genetic correlation attributable to common variation were estimated using LDAK (v5.1), under the baseline linkage disequilibrium (BLD)-LDAK and LDAK-Thin models, respectively.43 As advocated, SNPs were filtered from respective GWAS on the basis of an INFO score > 0.9, MAF > 0.01, harmonized to HapMap3 with 1000 Genomes European MAF > 0.05, excluding indels, structural variants, and strand-ambiguous SNPs. Transformation of observed scale heritability estimates of glioma to the liability scale was carried out assuming a lifetime risk of 0.24%, 0.13%, and 0.11% for all, GBM, and non-GBM glioma, respectively.44,45 $P$-values were estimated using a WaldTest (Supplementary Table 5).

Results

A significant relationship between genetically increased LTL and an increased risk of glioma was observed under an IVW-RE model; ORs per SD of 4.79 (95% CI: 2.11-10.85; $P = 1.76 \times 10^{-4}$), 5.46 (95% CI: 1.84-16.20; $P = 2.23 \times 10^{-3}$), and 4.24 (95% CI: 2.38-7.55, $P = 9.56 \times 10^{-7}$) were estimated for all glioma, GBM, and non-GBM glioma, respectively (Figure 2A-C). These associations were robustly significant across a number of different models (Supplementary Table 2). Leave-one-out analysis results confirmed that no single variant was responsible for the strong associations.

**Fig. 1** Overview of study design. Solid arrows represent a possible mechanism by which genetic variation influencing leukocyte telomere length (LTL) is associated with glioma risk. Dashed arrows correspond to summary Mendelian randomization analyses.
### Table 1: Summary Mendelian Randomization Analysis Using LTL-Associated Variants and Glioma GWAS Data

| Locus (Gene) | SNP | OR (95%CI) | P     |
|--------------|-----|------------|-------|
| 5p15 (TERT)  | rs7705526 | 76.57 (45.71–128.26) | 5.0 x 10^{-4} |
| 20q13 (RTET) | rs34978822 | 23.68 (7.99–70.16) | 1.1 x 10^{-3} |
| 5p15 (TERT)  | rs7705526 | 7.54 (2.94–19.37) | 2.7 x 10^{-3} |
| 20q13 (RTET) | rs34978822 | 6.14 (2.83–13.28) | 4.1 x 10^{-3} |
| 5p15 (TERT)  | rs7705526 | 5.00 (1.64–15.21) | .005   |
| 20q13 (RTET) | rs34978822 | 4.45 (1.45–13.61) | .009   |
| 5p15 (TERT)  | rs7705526 | 4.14 (1.60–10.71) | .003   |
| 20q13 (RTET) | rs34978822 | 3.91 (1.13–13.45) | .031   |
| 5p15 (TERT)  | rs7705526 | 2.99 (1.87–4.79) | 5.3 x 10^{-4} |
| 20q13 (RTET) | rs34978822 | 2.45 (0.56–11.00) | .229   |
| 5p15 (TERT)  | rs7705526 | 1.96 (0.64–6.04) | .241   |
| 20q13 (RTET) | rs34978822 | 1.89 (0.91–3.93) | .090   |
| 5p15 (TERT)  | rs7705526 | 0.75 (0.28–1.99) | .563   |
| 20q13 (RTET) | rs34978822 | 0.74 (0.24–2.26) | .595   |
| 5p15 (TERT)  | rs7705526 | 0.40 (0.11–1.46) | .164   |
| 20q13 (RTET) | rs34978822 | 0.07 (0.02–0.25) | 4.1 x 10^{-5} |

### Fig. 2: Forest plot showing the effect of alleles associated with longer leukocyte telomere length on risk of (A) all glioma, (B) GBM only, and (C) non-GBM only. Diamonds represent overall causal effects estimated using random-effects inverse-variance-weighted (IVW-RE) models. Confidence intervals (CIs) are indicated by diamond width. The vertical line denotes the null value (OR SD = 1). Abbreviations: GBM, glioblastoma multiforme; ORSD, ORs per 1 SD unit increase in the putative risk factor.
With the exception of \( P = 5.45 \times 10^{-3} \), 7q31.33 (rs59294613; values of \( P_{\text{het}} = 1.78 \times 10^{-37} \)) of heterogeneity existed among the SNPs used as IVs with \( RTEL1 \), \( TERT \), \( STN1 \) (alias \( OBFC1 \)), 10q24.33, 16q23, 19p12, and 20q13 loci were consistent with the associations reported to support 2 independent LTL-glioma associations being primarily driven by GBM (Supplementary Table 7), while the 6p21.33, 7q31.33, and 10q24.33 associations relate to non-GBM tumors (Supplementary Table 7).

At the 1q42.12, 3q26.2, 6p21.33, 6p22.2, 7q31.33, and 10q24.33 loci, the association signals for LTL and glioma risk were concordant—HEIDI test results consistent with causal associations (ie, pleiotropic with both phenotypes being influenced by the same genetic variant) (Table 1; Supplementary Table 6 and Supplementary Figure 1). In contrast, HEIDI test results for 5p15.33, 11q22.3, and 20q13.33 loci were consistent with the associations being primarily driven by linkage (ie, genetic variants in LD independently influencing phenotypes) (Table 1; Supplementary Table 6 and Supplementary Figure 1).

The strongest LTL association for glioma was provided by the 5p15.33 SNPs rs7705526 and rs2853677. There is LD between these SNPs, albeit not particularly strong \( (\rho = .16, D = 0.48) \), and conditional analysis has been reported to support 2 independent LTL association signals. Both associations for glioma are, however, several orders of magnitude weaker than that afforded by the GWAS glioma sentinel SNP rs10069690 \( (P = 5.01 \times 10^{-61} \text{ and } 1.08 \times 10^{-28}) \). Conditional analysis of glioma GWAS data does not support the existence of an additional association to that provided by rs10069690. Hence, the results of the HEIDI analysis are consistent with the findings from the GWAS.

### Table 1: Summary Mendelian Randomization Analysis Using LTL-Associated Variants and Glioma GWAS Data

| Locus | SNP        | Proximal Gene | \( P_{\text{Glioma}} \) | \( P_{\text{LTL}} \) | \( P_{\text{SMR}} \) | \( q_{\text{SMR}} \) | \( q_{\text{HEIDI}} \) |
|-------|------------|---------------|--------------------------|----------------------|----------------------|-----------------------|------------------------|
| 5p15  | rs7705526  | TERT          | \( 5.01 \times 10^{-61} \) | \( 5.34 \times 10^{-45} \) | \( 9.80 \times 10^{-27} \) | \( 1.86 \times 10^{-25} \) | \( 1.71 \times 10^{-3} \) |
| 5p15  | rs2853677  | TERT          | \( 1.08 \times 10^{-28} \) | \( 3.35 \times 10^{-31} \) | \( 9.68 \times 10^{-16} \) | \( 9.20 \times 10^{-15} \) | \( 3.88 \times 10^{-8} \) |
| 3q26  | rs10936600 | TERC          | \( 5.34 \times 10^{-6} \)  | \( 7.18 \times 10^{-51} \) | \( 1.33 \times 10^{-5} \)  | \( 8.42 \times 10^{-5} \)  | \( .871 \)             |
| 20q13 | rs34978822 | RTEL1         | \( 1.12 \times 10^{-8} \)  | \( 7.26 \times 10^{-10} \) | \( 2.81 \times 10^{-5} \)  | \( 1.34 \times 10^{-4} \)  | \( 7.86 \times 10^{-8} \) |
| 10q24 | rs9419958  | STN1 (OBFC1)  | \( 4.15 \times 10^{-6} \)  | \( 5.05 \times 10^{-19} \) | \( 4.31 \times 10^{-5} \)  | \( 1.64 \times 10^{-4} \)  | \( .160 \)             |
| 20q13 | rs75691080 | RTEL1/STM3N3  | \( 2.67 \times 10^{-5} \)  | \( 5.99 \times 10^{-14} \) | \( 2.47 \times 10^{-4} \)  | \( 7.82 \times 10^{-4} \)  | \( 3.21 \times 10^{-3} \) |
| 11q22 | rs228595   | ATM           | \( 4.09 \times 10^{-5} \)  | \( 1.43 \times 10^{-8} \)  | \( 8.89 \times 10^{-4} \)  | \( 2.41 \times 10^{-3} \)  | \( 2.56 \times 10^{-3} \) |
| 6p22  | rs34991172 | CARMIL1       | \( 1.55 \times 10^{-3} \)  | \( 6.19 \times 10^{-9} \)  | \( 5.45 \times 10^{-3} \)  | \( 1.29 \times 10^{-2} \)  | \( .829 \)             |
| 7q31  | rs59294613 | POT1          | \( 3.46 \times 10^{-3} \)  | \( 1.17 \times 10^{-13} \) | \( 6.52 \times 10^{-3} \)  | \( 1.38 \times 10^{-2} \)  | \( .767 \)             |
| 6p21  | rs2736176  | PRRC2A        | \( 4.57 \times 10^{-3} \)  | \( 3.53 \times 10^{-10} \) | \( 9.76 \times 10^{-3} \)  | \( 1.85 \times 10^{-2} \)  | \( .449 \)             |
| 1q42  | rs2255403  | PARP1         | \( 8.92 \times 10^{-3} \)  | \( 1.72 \times 10^{-10} \) | \( 1.55 \times 10^{-2} \)  | \( 2.68 \times 10^{-2} \)  | \( .711 \)             |
| 14q24 | rs2302588  | DCAF4         | \( 3.09 \times 10^{-2} \)  | \( 1.68 \times 10^{-8} \)  | \( 4.38 \times 10^{-2} \)  | \( .069 \)              | \( .409 \)             |
| 4q32  | rs4691895  | NAF1          | \( .089 \)                | \( 1.58 \times 10^{-21} \) | \( .095 \)               | \( .139 \)              | \( .291 \)             |
| 20q13 | rs73624724 | RFWD3         | \( .138 \)                | \( 6.33 \times 10^{-12} \) | \( .147 \)               | \( .200 \)              | \( 2.35 \times 10^{-46} \) |
| 16q23 | rs62053850 | TERF2         | \( .164 \)                | \( 4.06 \times 10^{-8} \)  | \( .178 \)               | \( .225 \)              | \( .139 \)             |
| 16q22 | rs3785074  | MOB1B         | \( .228 \)                | \( 2.43 \times 10^{-8} \)  | \( .240 \)               | \( .265 \)              | \( .335 \)             |
| 4q13  | rs13173667 | MPHOSPH6      | \( .594 \)                | \( 6.94 \times 10^{-10} \) | \( .596 \)               | \( .596 \)              | \( .494 \)             |
| 16q23 | rs7194734  | ZNF208        | \( .563 \)                | \( 5.42 \times 10^{-13} \) | \( .565 \)               | \( .596 \)              | \( .430 \)             |

**Abbreviations:** GWAS, genome-wide association studies; HEIDI, heterogeneity in dependent instruments; LTL, leukocyte telomere length; SMR, summary Mendelian randomization.

\( P_{\text{LTL}} \) published \( P \)-value. Complete results are detailed in Supplementary Table 6.
compatible with the LTL associations for glioma being a consequence of linkage at this locus, presumably reflecting the LD with the glioma risk SNP rs10069690 (LD with rs7705526: $r^2 = .33$, $D' = 0.63$, which in turn is in LD with rs2853677: $r^2 = .16$, $D' = 0.48$).

A similar scenario may also apply to the 20q13 associations for glioma with LTL SNPs rs75691080 ($P = 2.67 \times 10^{-6}$ and rs34978822 ($P = 1.12 \times 10^{-4}$). The LTL SNPs are acting as weak proxies for the GWAS glioma risk SNP rs2297440 ($P = 2.53 \times 10^{-42}$). The modest association $P$-values may be reflective of the limited LD with rs2297440 (rs75691080, $r^2 = .023$, $D' = 1.0$ and rs34978822, $r^2 = .051$, $D' = 1.0$).

Haplotype fine-mapping, centered on each of the LTL-associated variants, revealed that 5 variants, which had shown an association with glioma risk, existed in haplotype blocks with variants associated with brain tissue-specific enhancer histone marks (Supplementary Table 8) at 10q24.33 (STN1 alias OBF1), 20q13.33 (RETL1), 1q42.21 (PARP1), 6p22.2 (CARMIL1), and 7q31.33 (POTT1). This observation lends weight to the proposition that significantly concordant variants are exerting their regulatory effect on both LTL and glioma in brain tissue.

To gain insight into a possible biological basis of the relationship between these LTL loci with glioma risk, we analyzed e-QTL data from peripheral whole blood (CAGE, n = 2765), and brain tissue (Brain eMeta, n = 1194), as well as brain me-QTL data (n = 1160), applying a similar SMR-based strategy as before (Tables 2-4 and Supplementary Tables 9-11). Among the significant relationships we identified, a number were especially noteworthy as they implicate genes with strong candidacy based on proximity and plausibility a priori (Supplementary Table 12), specifically, (1) me-QTLs—5q15.33 (TERT), 6p21.3 (PRC2A, BAG6, FLOT1), and 7q31.33 (POTT1); (2) e-QTL—20q13.33 (STMN3); (3) e-QTL and me-QTL—1q42.12 (PARP1), 10q24.33 (STN1/OFBC1), ITRIP, GSTO2), and 11q22.3 (ATM/NPAT1).

The above analyses exploring the relationship between telomere genetics and glioma risk have been restricted to examining the LTL genome-wide significant SNPs. To explore the inter-relationship between heritable variation in telomeres and glioma risk on a genome-wide basis, we conducted a cross-trait LDAK analysis. The heritability of the glioma and LTL associated with all common genetic variation was 17.2% (SD: 1.6%), $P$.
Table 3  Relationship Between Gene Expression (Brain e-QTL) With Leukocyte Telomere Length (LTL) SNPs

| LTL Locus | Proximal Gene | SNP      | \( P_{\text{LTL}} \) | \( P_{\text{eQTL}} \) | \( q_{\text{SMR}} \) | \( P_{\text{SMR}} \) | \( P_{\text{HEIDI}} \) |
|-----------|---------------|----------|-----------------|-----------------|----------------|----------------|----------------|
| 6p21      | BAG6          | rs2523593| 4.28 x 10^{-7}   | 2.14 x 10^{-287}| 5.51 x 10^{-7} | 5.95 x 10^{-5} | .011          |
| 6p21      | C4A           | rs501942 | 4.90 x 10^{-6}   | 9.49 x 10^{-48} | 1.31 x 10^{-5} | 4.72 x 10^{-6} | 4.65 x 10^{-3} |
| 6p22      | BTN3A2        | rs9366653| 8.34 x 10^{-6}   | <1 x 10^{-302}  | 9.59 x 10^{-6} | 5.18 x 10^{-4} | 4.92 x 10^{-4} |
| 6p21      | FLOT1         | rs2596495| 7.39 x 10^{-7}   | 4.53 x 10^{-17} | 2.00 x 10^{-5} | 5.40 x 10^{-4} | .133          |
| 6p21      | CCHCR1        | rs2523593| 4.28 x 10^{-7}   | 1.13 x 10^{-10} | 6.92 x 10^{-5} | 1.50 x 10^{-3} | 2.94 x 10^{-3} |
| 11q22     | ATM           | rs10890822| 4.22 x 10^{-7}   | 1.71 x 10^{-9}  | 1.07 x 10^{-4} | 1.93 x 10^{-3} | 6.11 x 10^{-4} |
| 6p21      | CYP21A1P      | rs6463   | 1.30 x 10^{-5}   | 2.92 x 10^{-13} | 1.82 x 10^{-4} | 2.81 x 10^{-3} | .362          |
| 6p22      | BTN3A3        | rs13220495| 2.29 x 10^{-5}   | 5.56 x 10^{-13} | 2.61 x 10^{-4} | 3.52 x 10^{-3} | .079          |
| 20q13     | ZBTB46        | rs6011149| 5.80 x 10^{-5}   | 6.21 x 10^{-15} | 3.52 x 10^{-4} | 4.22 x 10^{-3} | 3.32 x 10^{-4} |
| 6p21      | DDAH2         | rs1144708| 1.86 x 10^{-5}   | 4.64 x 10^{-10} | 4.18 x 10^{-4} | 4.51 x 10^{-3} | .039          |
| 6p21      | VARS2         | rs2507989| 6.69 x 10^{-4}   | 1.26 x 10^{-17} | 1.57 x 10^{-3} | 1.54 x 10^{-2} | .227          |
| 3q26      | AC008404.01   | rs13315158| 2.96 x 10^{-4}   | 3.03 x 10^{-8}  | 2.45 x 10^{-3} | 2.20 x 10^{2}  | .435          |
| 10q24     | STN1          | rs3850670| 5.55 x 10^{-4}   | 8.50 x 10^{-9}  | 3.06 x 10^{-3} | 2.55 x 10^{-2} | .021          |
| 20q13     | STMN3         | rs601016 | 1.40 x 10^{-3}   | 1.74 x 10^{-10} | 4.28 x 10^{-3} | 3.30 x 10^{-2} | .230          |
| 10q24     | GSTO2         | rs276203 | 6.59 x 10^{-3}   | <1 x 10^{-302}  | 6.71 x 10^{-3} | 4.83 x 10^{-2} | .494          |

**Abbreviations**: e-QTL, expression quantitative trait loci; HEIDI, heterogeneity in dependent instruments; SMR, summary Mendelian randomization.

*Analysis +/- 1 Mb around LTL SNPs. Only significant e-QTL probes (FDR \( q < .05 \)) are shown.

Complete results are detailed in Supplementary Table 10.

Discussion

Our analysis is predicated on the assumption that any genetic variation influencing telomere length will have a generic component, and hence LTL will be correlated with telomere length in the presumptive glioma cell and/or tissue of origin. The LD patterns that are present at some loci, such as 20q13 (RTEL1), mean that 2 variants in LD with one another may exert their regulatory effect in different tissues, with the lead glioma risk SNP (rs10069690) having the strongest regulatory effect in glial cells, whereas the LTL lead SNPs (rs14978822/rs75691080) having the strongest regulatory effect in leukocytes.\(^{46}\) Fine-mapping of the LTL-associated variant haplotype blocks revealed that 5 LTL loci (PARP1, CARML1, POT1, STN1, and RTEL1) existed in haplotype blocks with variants that were known to have enhancer histone marks in brain tissue, further strengthening the assumption that this study is based on.

This study provides additional support for genetically predicted longer telomeres being the basis for glioma predisposition at a subset of the risk loci. As well as confirming a number of the previously reported LTL associations at 3q26.2, 5p15.33, 10q24.33, and 20q13.33, our analysis implicates variation at 1q42.12, 6p21.3, 6p22.2, and 7q31.33 as risk factors for glioma risk. Perhaps not surprisingly, the strongest associations observed were those mapping to the known glioma GWAS risk loci, with implicated target genes having well-established roles in telomere biology and glioma oncogenesis—5p15.33 (TERT), 3q26.2 (TERC), 10q24.33 (STN1, formerly OBFC1), and 20q13.33 (RTEL1). In addition, our analysis implicates variation at 1q42.12, 6p21.3, 6p22.2, 7q31.33, and 11q22.3 as risk factors for glioma. We and others have previously shown that glioma risk loci often exhibit associations with particular molecular subtypes, particularly based on combinations of IDH mutation (IDH1 or IDH2), 1p/19q co-deletion, and TERT promoter mutation.\(^{5,47}\) It has been reported that the majority of grade IV gliomas (ie, GBM tumors) contain TERT promoter mutations, and most grades II and III gliomas contain IDH mutations.\(^{5,48}\) Therefore, future large-scale glioma association studies stratifying by molecular subgroups are likely to provide increased insight into the nature of the relationship between glioma risk polymorphisms and telomere maintenance mechanisms. Indeed, we note that SNP associations at telomere-related loci 3q26 (TERC), 5p15.33 (TERT), and 20q13.33 (RTEL1) show a consistent direction of effect with those in our study for GBM glioma and in the analysis of Eckel-Passow \textit{et al.}\(^{5}\) for gliomas with TERT promoter mutation.

The correlation between LTL and telomere length in brain tissue is imperfect.\(^{49}\) Despite this limitation, 3 of the prioritized genes mapping to the newly identified glioma risk loci on the basis of LTL association have recognized roles in regulation of telomeres—\textit{PARP1} (1q42.12), \textit{POT1} (7q31.33), and \textit{ATM} (11q22.3). Telomeres function to prevent the 3’ single-stranded overhang at the end of the chromosome from being detected as a double-stranded DNA break. This is achieved through binding of the Shelterin complex (TERF1, TERF2, TERF2IP, TINF2, ACD, and POT1), which acts to block activation of DNA damage response (DDR) pathways. Shelterin also binds a number of accessory factors that

\(= 5.93 \times 10^{-27}\) and 3.1% (SD: 1.2%, \( P = 9.79 \times 10^{-3}\)), respectively, with 0.8% (SD: 0.9%, \( P = .35\)) co-heritability (Supplementary Table 5).
have been reported to be associated with familial oligo-
intriguingly, inactivating germline mutations in
STN1
(formerly OBFC1
RTEL1
POT1
POT1
, the helicase
), shelterin also interacts with the CST complex that regulates telomerase access to the telomeric DNA.\textsuperscript{50} The associated loci contain one of the shelterin components
ATM
, and the
CST
component
POT1
.\textsuperscript{50} ATM
is required for telomere elongation, possibly through
homologous end joining at telomeres,\textsuperscript{51}\textsuperscript{52} activation and the classical non-
inhibits the binding of
TERF2
, whose expression is linked to DNA damage signaling and apoptosis is a strong candidate a priori.\textsuperscript{53} Although telomere-binding proteins and structure play a role in influencing telomere biology (for 80% power required genetic correlation needing to be > 0.12).\textsuperscript{57} It is, however, the case that a number of the associations and the genes that they annotate support the role of genetically determined telomere dysfunction as being biologically important in glioma. Excluding
TERT
, there is effectively no overlap between genes related to the respective susceptibility loci and somatic mutated genes in glial tumors suggesting that they each may act at different moments in the development of the tumourigenesis. Finally, where appropriate, this analysis serves to illustrate the value of hypothesis-driven discovery as an adjunct to conventional agnostic GWAS-based approaches to risk factor identification.

| LTL Locus | Proximal Gene | SNP | \(P \)\textsubscript{LTL} | \(P \)\textsubscript{LTL} | \(P \)\textsubscript{SMR} | \(q \)\textsubscript{SMR} | \(R \)\textsubscript{HEIDI} |
|-----------|--------------|-----|----------------|----------------|----------------|----------------|----------------|
| 5p15      | TERT         | rs2736100  | 2.28 x 10^{-41} | 1.20 x 10^{-33} | 2.30 x 10^{-19} | 1.48 x 10^{-16} | .106 |
| 20q13     | ZBTB46       | rs58150746  | 7.18 x 10^{-10} | 2.79 x 10^{-78} | 4.82 x 10^{-9}  | 8.46 x 10^{-7}  | .053 |
| 1q42      | PARP1        | rs2048425   | 1.92 x 10^{-10} | 8.04 x 10^{-25} | 6.15 x 10^{-8}  | 6.60 x 10^{-6}  | .153 |
| 6p21      | LY6G6C       | rs707930    | 7.43 x 10^{-8}  | 1.00 x 10^{-300} | 1.01 x 10^{-7}  | 8.88 x 10^{-6}  | .018 |
| 6p21      | PRRC2A       | rs3091280   | 1.71 x 10^{-7}  | 7.51 x 10^{-67} | 6.83 x 10^{-7}  | 4.71 x 10^{-5}  | .028 |
| 7q31      | POT1         | rs4311608   | 1.32 x 10^{-7}  | 1.17 x 10^{-19} | 5.10 x 10^{-6}  | 2.47 x 10^{-4}  | .265 |
| 6p21      | FLOT1        | rs9262132   | 8.47 x 10^{-7}  | 7.72 x 10^{-20} | 1.06 x 10^{-5}  | 4.09 x 10^{-4}  | .146 |
| 6p21      | DDAH2        | rs707916    | 2.62 x 10^{-5}  | <1 x 10^{-307} | 2.88 x 10^{-5}  | 7.23 x 10^{-4}  | .012 |
| 6p21      | VARS2        | rs1264309   | 9.68 x 10^{-6}  | 2.84 x 10^{-290} | 1.06 x 10^{-4}  | 1.47 x 10^{-3}  | .011 |
| 6p21      | HLA-C        | rs2524074   | 5.47 x 10^{-5}  | 4.22 x 10^{-42} | 1.10 x 10^{-4}  | 1.48 x 10^{-3}  | .018 |
| 11q22     | ATM           | rs624888    | 5.84 x 10^{-7}  | 1.69 x 10^{-9}  | 1.20 x 10^{-4}  | 1.54 x 10^{-3}  | .258 |
| 20q13     | LIME1        | rs6011058   | 6.98 x 10^{-5}  | 2.44 x 10^{-40} | 1.39 x 10^{-4}  | 1.70 x 10^{-3}  | .039 |
| 6p21      | HSPA1A       | rs2763979   | 8.40 x 10^{-6}  | 1.45 x 10^{-12} | 1.63 x 10^{-4}  | 1.91 x 10^{-3}  | .123 |
| 10q24     | STN1         | rs11191841  | 1.85 x 10^{-6}  | 1.78 x 10^{-9}  | 1.86 x 10^{-4}  | 2.05 x 10^{-3}  | .052 |
| 6p21      | CCHCR1       | rs7750641   | 4.98 x 10^{-6}  | 8.85 x 10^{-10} | 2.51 x 10^{-4}  | 2.47 x 10^{-3}  | .027 |
| 6p21      | HCP5         | rs2844503   | 2.43 x 10^{-4}  | 5.04 x 10^{-14} | 9.71 x 10^{-4}  | 6.92 x 10^{-3}  | .032 |
| 6p21      | BAG6         | rs3130047   | 3.44 x 10^{-4}  | 1.77 x 10^{-13} | 1.28 x 10^{-3}  | 8.65 x 10^{-3}  | .051 |
| 20q13     | UCKL1        | rs7264220   | 1.29 x 10^{-3}  | 2.43 x 10^{-11} | 3.74 x 10^{-3}  | 2.24 x 10^{-2}  | .455 |
| 10q24     | ITPRIP       | rs34970111  | 1.99 x 10^{-3}  | 1.26 x 10^{-10} | 5.32 x 10^{-3}  | 3.02 x 10^{-2}  | .075 |
| 10q24     | GSTO2        | rs276204    | 6.09 x 10^{-3}  | <1 x 10^{-307} | 6.23 x 10^{-3}  | 3.48 x 10^{-2}  | .122 |
| 11q22     | ACAT1        | rs35219733  | 5.43 x 10^{-3}  | 9.86 x 10^{-17} | 8.37 x 10^{-3}  | 4.40 x 10^{-2}  | .033 |

**Abbreviations:** HEIDI, heterogeneity in dependent instruments; me-QTL, methylation quantitative trait loci; SMR, summary Mendelian randomization.

\*Analysis shows only the most significantly associated probe results, shown by the HEIDI test to be truly causal or pleiotropic, at genes that had a significant concordant association signal in the previous analyses. Complete results are detailed in Supplementary Table 11.

Supplementary Material

Supplementary material is available online at Neuro-Oncology.

Keywords

glioma | Mendelian randomization | risk factors | telomere length
Keywords...
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