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Telomere Length and Risk of Glioma

Farzana Walcott^a, Preetha Rajaraman^{b,c}, Shahinaz M. Gadalla^d, Peter D. Inskip^c, Mark P. Purdue^e, Demetrius Albanes^f, Esther Orr^g, Immaculata De Vivo^{g,h}, and Sharon A. Savage^{d,*}

^a Cancer Prevention Fellowship Program, Division of Cancer Prevention, National Cancer Institute, National Institutes of Health, Rockville, MD 20892

^b Center for Global Health, National Cancer Institute, National Institutes of Health, Rockville, MD 20892

^c Radiation Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Rockville, MD 20892

^d Clinical Genetics Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Rockville, MD 20892

^e Occupational and Environmental Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Rockville, MD 20892

^f Nutritional Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Rockville, MD 20892

^g Channing Laboratory, Department of Medicine, Brigham and Women's Hospital/Harvard Medical School, Boston, MA 02115

^h Department of Epidemiology, Harvard School of Public Health, Boston, MA 02115

Abstract

Background—Telomere length in blood or buccal cell DNA has been associated with risk of various cancers. Glioma can be a highly malignant brain tumor and has few known risk factors. Genetic variants in or near *RTEL1* and *TERT*, key components of telomere biology, are associated with glioma risk. Therefore, we evaluated the association between relative telomere length (RTL) and glioma in a prospective study.

Materials and Methods—We performed a nested case-control study within the Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial. RTL was determined by quantitative PCR on blood or buccal cell DNA obtained at least two years prior to diagnosis from 101 individuals with glioma cases. Healthy controls (n=198) were matched to cases (2:1) on age, gender, smoking status, calendar year, and DNA source. Conditional logistic regression was used to investigate the association between RTL and glioma.

Results—As expected, RTL declined with increasing age in both cases and controls. There was no statistically significant association between RTL and glioma overall. An analysis stratified by

*Corresponding Author Sharon A. Savage, M.D., F.A.A.P. Senior Investigator Clinical Genetics Branch Division of Cancer Epidemiology and Genetics National Cancer Institute 6120 Executive Blvd. EPS 7018 Rockville, MD 20892 Tel: 301-496-5785 savagesh@mail.nih.gov.

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Conflicts of interest

The authors have no potential conflicts of interest.

gender suggested that short RTL (1st tertile) in males was associated with glioma (odds ratio, [OR] = 2.29, 95% confidence interval [CI] 1.02-5.11); this association was not observed for females (OR=0.41, 95% CI 0.14-1.17).

Conclusions—This prospective study did not identify significant associations between RTL and glioma risk, but there may be gender-specific differences. Larger, prospective studies are needed to evaluate these findings.

Keywords

Telomere length; glioma; epidemiology; cancer risk

1. INTRODUCTION

Telomere length in surrogate tissues (*e.g.*, blood or buccal cells) has been associated with cancer risk in several studies[1, 2]. Telomeres are specialized nucleoprotein structures at chromosome ends that are critical for chromosomal stability. They shorten with each cell division and when critically short length is attained, cellular senescence or apoptosis is triggered[3]. Cancer cells continue to divide in the presence of short telomeres by up-regulating key pathways, such as telomerase (*TERT*) or alternative lengthening of telomeres (ALT)[3].

Gliomas account for approximately 69% of malignant brain tumors in the United States [4]. The five-year survival rate for all subtypes of glioma in the U.S. from 2001-2008 was approximately 45%. High grade gliomas, such as glioblastoma multiforme, have a much worse outcome with a five-year survival rate of approximately 2.9%[5]. Gliomas are more common in males than in females[4, 6]. Confirmed risk factors for glioma include moderate-to-high doses of ionizing radiation, certain inherited cancer predisposition syndromes, and a family history of glioma[7].

Genome-wide association studies (GWAS) of glioma have identified common susceptibility variants at seven loci, including two telomere biology genes, *TERT* and *RTEL1*[7-10]. Further, a recent study found increasing numbers of risk alleles in *TERT* and *RTEL1* genes were associated with increased risk of glioma in older patients, while increasing numbers of risk alleles in *CCDC26* and *PHLDB1* genes was associated with younger age of diagnosis of glioma [11]. The association between telomere length and SNPs in *TERT* or *RTEL1* is not clear; some studies have found associations whereas others have not [12, 13].

There is a growing connection between changes in telomere biology in somatic glioma tissues, including *TERT* promoter mutations in 83% of glioblastoma multiforme [14]. Telomere length heterogeneity appears to be present in brain tumors. Some brain tumors appear to up-regulate telomerase whereas others maintain telomere length through the alternative lengthening of telomere pathway (ALT) [15-20]. The ALT pathway may play a role in specific subsets of glioma with mutations in Alpha Thalassemia/Mental Retardation X-linked (ATRX) and/or isocitrate dehydrogenase genes (IDH1) [18].

In order to better understand the connection between germline telomere biology and glioma, we conducted a nested case-control study within the Prostate, Lung, Colorectal, and Ovarian Cancer (PLCO) Screening Trial.

2. METHODS

2.1 Study Design

Our case-control study was nested within the prospective PLCO Screening Trial [21]. All study participants were of European ancestry, cancer-free at enrollment, aged between 55-75 years, enrolled between 1993 and 2001, and followed until at least 2010. For our study, cases were individuals who developed glioma as a first cancer between 1995-2010. Controls were cancer-free at time of case diagnosis. Cases and controls (two per case) were matched on: trial randomization arm (control arm or intervention arm), gender, age at baseline in 4 categories (55-59, 60-64, 65-69, 70+), calendar year of randomization, cigarette smoking status (never, current, former), and calendar year of blood draw. In addition, cases and controls were fully matched on DNA source except for 17 cases where matching was for only one control. In order to minimize the possibility of reverse causality association, only biological specimens that were collected two or more years prior to diagnosis/selection were considered.

2.2 Laboratory Methods

DNA was extracted by standard methods (phenol:chloroform or Qiagen kits) from blood, buffy coat, or buccal cells. The average relative telomere length (RTL) was determined using qPCR as described previously [22, 23]. RTL was calculated as the telomere repeat copy number/single-gene (*36B4*) copy number (T/S) exponentiated ratio. Laboratory technicians were blinded to the case-control status. All assays were performed in triplicate on 5 ng of genomic DNA. Forty blinded, randomly selected, quality control samples were interspersed throughout the dataset in order to assess inter-plate and intra-plate variability. The coefficient of variation (CV) for triplicate of the telomere copy (inter-assay) was 0.9% and for the single-copy gene was 0.8%. The CV for duplicate quality control samples (intra-assay) was 3.92% with a range of 0.42%-7.82%.

2.3 Statistical Analysis

Spearman's rank correlation was used to assess correlations between RTL and age for cases and controls. We used Wilcoxon-rank test to compare median RTL in cases and controls overall, and by subgroup. Conditional logistic regression was used to examine the association between age-adjusted RTL and glioma. Stratified analyses by gender were performed to assess the interaction of gender and RTL on glioma risk. In all analyses, RTL was assessed as a continuous variable and in tertiles (based on the distribution in the controls). The final analysis included 198 controls and 101 cases (RTL data were not available on 12 controls and 4 cases due to qPCR assay failures). All analyses were performed using SPSS version 19. All tests were two-sided.

3. RESULTS

Per study design, the glioma cases and controls were similar in regard to age distribution, gender, and smoking status (Table 1). There were more males than females with glioma (male:female ratio 3:2). RTL was inversely correlated with age in the controls ($r = -0.14$, $p = 0.04$). The RTL-age correlation in the cases was not statistically significant ($r = 0.10$, $p = 0.34$). The median RTL in glioma cases and controls was not significantly different (median RTL in cases = 0.74 and in controls = 0.77, $p = 0.84$). In the regression model, the evaluation of risk of glioma and RTL as both a continuous variable and in tertiles of RTL in controls revealed no statistically significant associations between RTL and overall risk of glioma (Table 2). Since RTL varies between tissues but is highly correlated within individuals we evaluated whether DNA sources affected our results [24-26]. Sensitivity

analyses adding DNA source to the conditional logistic regression model made no difference (data not shown).

Case-case comparison of RTL showed that males with glioma had shorter telomeres than females with glioma (male cases median RTL = 0.63, female cases median RTL = 0.91, $p = 0.02$) (Table 1). This relationship between RTL and gender was not observed in controls. However, gender-specific analyses suggested an association between RTL and glioma in males (continuous RTL $p = 0.05$ and shortest RTL tertile/longest OR = 2.29, 95% CI 1.02-5.11).

4. Discussion

The etiology of glioma is not well understood, although genetic factors, high levels of exposure to ionizing radiation, and male gender appear to be risk factors[7]. We conducted a case-control study of RTL in glioma because there is a growing body of evidence suggesting that aberrant telomere biology may be associated with glioma. Common germline variants in key telomere biology genes, *RTEL1* and *TERT*, are associated with glioma risk [7-10]. Several studies have found a connection between telomerase levels, telomere heterogeneity, *TERT* promoter mutations and ALT in somatic glioma tissues and provide further evidence for a biological connection [14] [15-20]. A growing number of studies suggest that short germline RTL is a risk factor for certain cancers, including bladder, esophageal, gastric, head and neck, ovarian, renal and overall incident cancer[1]. However, many of these studies were limited by sample size and by their retrospective case-control nature. Recent prospective studies suggest that germline RTL may not be strongly associated with cancer risk but, instead, may be associated with early mortality [27, 28].

Evaluations of “brain and nervous tissue cancer” and RTL were included, to a limited degree, in a large prospective study of leukocyte RTL in 3,142 individuals with cancer from a cohort of 47,102 participants in two Danish cohorts [27]. That study included 65 individuals with brain and nervous tissue cancer. There were no statistically significant associations between RTL and risk of brain and nervous tissue cancer. However, the risk of early death after brain and nervous tissue cancer was increased in individuals with shorter telomeres (hazard ratio 1.52, 95% CI 1.00-2.31) [27].

Our study is the first to specifically evaluate RTL in prospectively collected buccal or leukocyte samples from glioma cases and controls. We did not find an association between overall glioma risk and germline RTL. However, our data suggest that males with short telomeres may be at increased risk of glioma compared with age-matched, cancer-free males. A larger sample size is required before drawing any firm conclusions.

A limitation of our study is that we were underpowered to stratify by tumor subtype. While subtypes of glioma may have different mechanisms of telomere maintenance, it is unclear if that would be reflected in a germline RTL study. Given the nature of glioma case and control sample availability in PLCO, our study is also limited because it included RTL measurements on blood and buccal cell DNA. We used sample source as a matching factor in the study design and performed sensitivity analyses that showed there was no difference in results based on sample source.

The strengths of our study include the well-characterized PLCO cohort, and its prospective nature with biologic samples collected at least two years prior to glioma diagnosis. In summary, our study suggests that germline RTL is not associated with overall glioma risk, but RTL may be associated with glioma in males. Larger studies of telomere biology and specific glioma subtypes are warranted to better understand its etiology and risk factors.

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Abbreviations

RTL	relative telomere length
PLCO	Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial
OR	odds ratio
CI	95% confidence interval
qPCR	quantitative PCR

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Table 1

Descriptive Characteristics and Median Telomere Length of Study Participants

	Cases N=102 (%)	Controls N=199 (%)	P value
Age			
55-59	27 (26)	50 (25)	0.90
60-64	31 (31)	61 (31)	
65-69	31 (31)	60 (30)	
70+	13 (13)	28 (14)	
Gender			
Males	62 (61)	122 (62)	0.90
Females	40 (40)	77 (39)	
Smoking status			
Never	54 (51)	105 (53)	0.99
Former	6 (6)	11 (6)	
Current	42 (42)	83 (42)	
	Median Relative Telomere Length (Range)	Median Relative Telomere Length (Range)	
All	0.74 (0.20-4.77)	0.77 [§] (0.14-3.72)	0.84
Males	0.63 (0.22-2.15)	0.77 [§] (0.14-3.72)	0.12
Females	0.91 ^{§§} (0.20-4.77)	0.77 [§] (0.16-3.56)	0.10
Male glioma cases vs. female glioma cases			0.02

Table 2

Age-adjusted association between relative telomere length and glioma

All participants					
	Cases N (%)	Controls N (%)	OR *	CI (95%)	P value
RTL (continuous)	102	193	0.84	0.55-1.27	0.84
RTL (median)	102	192	1.17	0.73-1.88	0.52 [§]
Tertiles of RTL					
Short (1 st tertile)	36 (36)	65 (33)	1.26	0.69-2.29	0.46
Medium (2 nd tertile)	32 (32)	67 (34)	1.06	0.55-2.04	0.86
Long (3 rd tertile)	33 (33)	66 (33)	1.00	REF	
Males only					
Continuous RTL	62	116	0.48	0.23-0.99	0.05
RTL (median)	62	116	1.74	0.93-3.24	0.081
Tertiles of RTL					
Short (1 st tertile)	29 (46)	38 (31)	2.29	1.02-5.11	0.04
Medium (2 nd tertile)	18 (30)	47 (38)	1.17	0.47-2.87	0.74
Long (3 rd tertile)	14 (22)	37 (30)	1.00	REF	
			P trend = 0.06		
Females only					
Continuous RTL	40	77	1.25	0.74-2.12	0.41
RTL (median)	40	76	0.64	0.30-1.37	0.25
Tertiles of RTL					
Short (1 st tertile)	7 (17)	27 (35)	0.41	0.14-1.17	0.09
Medium (2 nd tertile)	14 (35)	20 (26)	1.21	0.45-3.28	0.70
Long (3 rd tertile)	19 (47)	29 (38)	1.00	REF	
			P trend = 0.15		

Abbreviations: RTL, relative telomere length; OR, odds ratio; CI, 95% confidence interval; N, number; REF, reference

* Conditional logistic regression with matching on age, gender, and smoking status

[§] DNA source added to conditional logistic regression model, OR= 1.52, 95% CI = 0.91-2.54, p=0.11