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The association between longer relative leukocyte telomere length and risk of glioma is independent of the potentially confounding factors allergy, BMI, and smoking

Ulrika Andersson¹ · Sofie Degerman² · Anna M. Dahlin¹ · Carl Wibom¹ · Gunnar Johansson¹ · Melissa L. Bondy³ · Beatrice S. Melin¹

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Abstract

Purpose Previous studies have suggested an association between relative leukocyte telomere length (rLTL) and glioma risk. This association may be influenced by several factors, including allergies, BMI, and smoking. Previous studies have shown that individuals with asthma and allergy have shortened relative telomere length, and decreased risk of glioma. Though, the details and the interplay between rLTL, asthma and allergies, and glioma molecular phenotype is largely unknown.

Methods rLTL was measured by qPCR in a Swedish population-based glioma case–control cohort (421 cases and 671 controls). rLTL was related to glioma risk and health parameters associated with asthma and allergy, as well as molecular events in glioma including *IDH1* mutation, 1p/19q co-deletion, and *EGFR* amplification.

Results Longer rLTL was associated with increased risk of glioma (OR = 1.16; 95% CI 1.02–1.31). Similar to previous reports, there was an inverse association between allergy and glioma risk. Specific, allergy symptoms including watery eyes was most strongly associated with glioma risk. High body mass index (BMI) a year prior diagnosis was significantly protective against glioma in our population. Adjusting for allergy, asthma, BMI, and smoking did not markedly change the association between longer rLTL and glioma risk. rLTL among cases was not associated with *IDH1* mutation, 1p/19q co-deletion, or *EGFR* amplification, after adjusting for age at diagnosis and sex.

Conclusions In this Swedish glioma case–control cohort, we identified that long rLTL increases the risk of glioma, an association not confounded by allergy, BMI, or smoking. This highlights the complex interplay of the immune system, rLTL and cancer risk.

Keywords Glioma · Relative leukocyte telomere length (rLTL) · Allergy · BMI · Smoking · IDH1 · 1p/19q · EGFR

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✉ Ulrika Andersson
ulrika.l.andersson@umu.se

¹ Department of Radiation Sciences, Oncology, Umeå University, Umeå, Sweden

² Department of Medical Biosciences, Pathology, Umeå University, Umeå, Sweden

³ Department of Medicine, Dan L. Duncan Comprehensive Cancer Center, Baylor College of Medicine, Houston, TX, USA

Introduction

Glioma, a malignant brain tumor confers a poor prognosis, and there are few established etiological factors. These include, rare inherited syndromes such as neurofibromatosis type I and II, LiFraumeni syndrome [1], and high dose ionizing radiation have been associated with increased risk of glioma development [2, 3]. Asthma and respiratory allergies have consistently been reported to be protective against glioma [4–7]. A number of common genetic variants have also been associated with glioma risk [8–10]. Both studies of familial and sporadic glioma have shown associations between glioma risk and genetic variants mapping to genes functionally important in telomere regulation, e.g., *RTEL*, *TERT*, *TERC* [8, 11], *POT1* [12], and *OBFC1* [13].

Telomeres, which are located at the chromosome ends, have important functions in sustaining cellular chromosomal stability. Since telomeres are shortened during replication, shorter telomere length is observed in aging tissues. Individuals with asthma in adulthood have shorter leukocyte telomeres compared to healthy controls [14]. Additionally, increased BMI and cigarette smoking are also known to be associated with shorter telomere length in white blood cells [15]. Short telomere length measured in surrogate tissues such as peripheral blood has been associated with increased risk of many diseases, including cardiovascular and respiratory diseases, as well as cancer [16]. Telomere length heterogeneity appears to be present in brain tumors, since a certain subtype of glioblastoma seems to up-regulate telomerase through highly clonal TERT promoter mutations, whereas other subtypes of glioblastoma maintain telomere length through subclonal mutations activating an alternative lengthening of telomeres pathway (ALT) [17]. Evidence suggests that there may be a link between glioma risk and telomere length, including the commonality that both telomere length and glioma have been associated with allergy and asthma.

IDH1 mutations and co-deletion of the 1p and 19q chromosomal arms (1p/19q co-deletion) are two of the main genetic biomarkers important for glioma classification and diagnosis [18]. A previously published study has shown that glioma with *IDH1* mutations and lack of 1p/19q co-deletion are negative for both hTERT expression and telomerase activity, and cases have significantly longer tumor tissue telomeres [19]. *EGFR* is frequently overexpressed in primary glioblastoma versus secondary glioblastoma and is a characteristic of a more aggressive phenotype. It has been shown that *EGFR* overexpression leads to phosphorylation of hTERT through the PI3K/AKT signaling pathway, resulting in the activation of telomerase [20]. Nevertheless, any potential link between rLTL in peripheral blood and glioma risk is not well characterized. Genotypically-estimated leukocyte telomere length is reported to be associated with glioma risk [21], but only a small number of studies and with contradictory results have measured rLTL in peripheral blood in glioma cases and controls [22, 23], reviewed in [24].

In this study, we investigated a population-based case–control series from Sweden with a quantitative-PCR method for rLTL, examining with direct measurement of the potential association between rLTL and risk of glioma. We controlled for age at diagnosis/enrollment, sex, allergy, asthma, smoking history, and BMI, as these are established factors associated with telomere length and, to some extent, glioma. Furthermore, we examined the association between rLTL, and molecular events in gliomagenesis, including *IDH1* mutation, 1p/19q co-deletion, and *EGFR* amplification.

Study subjects and methods

Ascertainment and collection of study subjects

We included all Swedish participants from two international multi-center efforts aimed at exploring glioma etiology: the Genetic Epidemiology of Glioma International Consortium (GLIOGENE) study [25], and the Glioma International Case–Control (GICC) study [26]. The GLIOGENE study included cases with hereditary disease diagnosed between 2007 and 2013 [25]. Cases with a reported or confirmed diagnosis of neurofibromatosis I, neurofibromatosis II, Turcot's syndrome, or tuberous sclerosis were excluded from the GLIOGENE study. The GICC study included sporadic cases diagnosed between 2010 and 2013, as well as population-based controls (aged 18–80, frequency matched to cases based on age at diagnosis and sex). The methods for each of these studies have been previously described [25, 26]. Study participants of GLIOGENE and GICC contributed biological samples and completed questionnaires. The GICC questionnaire included questions related to allergies, asthma, smoking history, and BMI. All sites received Institutional Review Board or ethical board approval to conduct the study, and informed consent was obtained from participants. Ultimately, we included 37 Swedish GLIOGENE-cases (36 probands and one affected relative) and 384 Swedish GICC-cases, as well as 671 population-based GICC-controls, and characteristics of the study subjects are shown in Table 1. For the analyses of certain subgroups of glioma, we used one or two age- and sex- matched controls for each case, since these glioma subgroups are known to be correlated with both age and sex (Table 2). From the questionnaires used for the GICC study, we extracted data on allergy status (experienced symptoms: skin/respiratory/watery eyes) and asthma (diagnosed by doctor) as well as two smoking variables: current smoking (yes/no) and ever smoking (yes/no), defined as “> 100 cigarettes in a lifetime” [26] (Table 1). We also extracted BMI, which was categorized as underweight/normal (BMI < 25) and overweight/obese (BMI ≥ 25) (Table 1). In collaboration with the Swedish GICC study centers, we obtained unstained slides from paraffin embedded tissue for 367 of the glioma patients. These slides were used in the immunohistochemistry (IHC) and fluorescent in situ hybridization (FISH) analyses (described below).

Relative telomere length (rLTL) PCR

Genomic DNA was extracted from the buffy coat from EDTA-venous blood samples using FlexiGene DNA Kit

Table 1 Characteristics of cases and matched controls identified through the GLIOGENE- and GICC-studies, and included in the relative telomere length analyses

	Controls <i>n</i> (%)	Cases all glioma cases <i>n</i> (%)	Low-grade glioma cases ^a <i>n</i> (%)	High-grade glioma cases ^a <i>n</i> (%)
<i>Sex</i>				
Male	400 (59.6)	250 (59.4)	87 (51.5)	162 (64.8)
Female	271 (40.4)	171 (40.6)	82 (48.5)	88 (35.2)
<i>Diagnosis/enrollment years</i>				
18–29 years	6 (0.9)	21 (5.0)	18 (10.6)	3 (1.2)
30–39 years	34 (5.0)	34 (8.0)	24 (14.2)	10 (4.0)
40–49 years	79 (11.8)	59 (14.0)	31 (18.3)	28 (11.2)
50–59 years	198 (29.5)	109 (25.9)	41 (24.3)	68 (27.2)
60–69 years	248 (37.0)	148 (35.2)	39 (23.1)	107 (42.8)
70–80 years	106 (15.8)	50 (11.9)	16 (9.5)	34 (13.6)
<i>Allergies (skin, respiratory, watery eyes, digestive problems, anaphylaxis, or other)</i>				
Yes	190 (28.3)	82 (19.5)	36 (21.3)	45 (18.0)
No	428 (63.8)	281 (66.7)	102 (60.4)	178 (71.2)
Missing	53 (7.9)	58 (13.8)	31 (18.3)	27 (10.8)
<i>Asthma (diagnosed by doctor)</i>				
Yes	64 (9.5)	35 (8.3)	15 (8.9)	19 (7.6)
No	599 (89.3)	348 (82.7)	136 (80.5)	211 (84.4)
Missing	8 (1.2)	38 (9.0)	18 (10.6)	20 (8.0)
<i>Ever smoking (smoked more than 100 cigarettes in life time)</i>				
Yes	363 (54.1)	207 (49.2)	76 (42.6)	129 (51.6)
No	299 (44.6)	168 (39.9)	72 (45.0)	96 (38.4)
Missing	9 (1.3)	46 (10.9)	21 (12.4)	25 (10.0)
<i>Current smoking (currently smoke cigarettes)</i>				
Yes	70 (10.4)	32 (7.6)	13 (7.7)	19 (7.6)
No	293 (43.7)	175 (41.6)	63 (37.3)	110 (44.0)
Missing	308 (45.9)	214 (50.8)	93 (55.0)	121 (48.4)
<i>BMI at age 18 (calculated as weight in kilograms divided by height in meters squared)</i>				
Valid	551 (82.1)	294 (69.8)	119 (70.4)	173 (69.2)
Missing	120 (17.9)	127 (30.2)	50 (29.6)	77 (30.8)
<i>BMI as adult (calculated as weight in kilograms divided by height in meters squared)</i>				
Valid	630 (93.9)	350 (83.1)	142 (84.0)	206 (82.4)
Missing	41 (6.1)	71 (16.9)	27 (16.0)	44 (17.6)
<i>BMI a year prior diagnosis (calculated as weight in kilograms divided by height in meters squared)</i>				
Valid	668 (99.6)	367 (87.2)	145 (85.8)	220 (88.0)
Missing	3 (0.4)	54 (12.8)	24 (14.2)	30 (12.0)

^aSum of low-grade and high-grade glioma cases does not equal the total number of cases, due to two cases with unclassified tumors

(Qiagen) and DNA purity and concentration were measured by the Nanodrop instrument (Thermo Fisher Scientific). rLTL was determined by the quantitative-PCR method described by Cawthon [27]. Briefly, Telomere (Tel) and single copy gene (HBG) reactions were run separately in triplicates. Tel/HBG (T/S) values were calculated by $2^{-\Delta C_t}$ method, where $\Delta C_t = \text{average } C_{t_{\text{Tel}}} - \text{average } C_{t_{\text{HBG}}}$. rLTL was generated by dividing a samples T/S value with the T/S value of a reference cell line DNA (CCRF) included in all runs. A standard curve generated by the reference DNA was also included in each run to

monitor PCR efficiency. The mean inter-assay coefficient of variation for rLTL in this study was 7%.

Immunohistochemistry (IHC)

Based on eosin staining, a neuropathologist reviewed all slides to ensure that there was adequate tumor tissue in each slide. Slides were pre-treated and stained against IDH1-R132H as previously described [28], using Ultra view DAB kit 760 (Roche, Basel Switzerland) and mouse anti-IDH1-R132H (Dianova, Hamburg, Germany). Slides

Table 2 Logistic regression models to investigate the association between rLTL and risk of glioma subgroups based on IDH1 mutation, 1p/19q co-deletion, and EGFR amplification. Glioma grade II-III are defined as non-GBM, and glioma grade IV are defined as GBM

	Cases ^a <i>n</i>	Controls ^b <i>n</i>	OR (95% CI) ^c	<i>P</i> value
rLTL in all glioma	321	598	1.15 (1.00–1.32)	0.046
rLTL in glioma with <i>IDH1</i> wildtype	265	501	1.16 (0.99–1.35)	0.067
rLTL in glioma with <i>IDH1</i> mutation and without 1p/19q co-deletion	25	40	1.48 (0.86–2.55)	0.155
rLTL in glioma with <i>IDH1</i> mutation and 1p/19q co-deletion	23	43	0.92 (0.55–1.56)	0.768
rLTL in glioma with <i>EGFR</i> amplification	100	192	1.11 (0.86–1.44)	0.423
rLTL in all non-GBM	122	211	1.13 (0.91–1.41)	0.259
rLTL in non-GBM with <i>IDH1</i> wildtype	75	131	1.28 (0.95–1.73)	0.108
rLTL in non-GBM with <i>IDH1</i> mutation and without 1p/19q co-deletion	20	30	1.31 (0.70–2.46)	0.400
rLTL in non-GBM with <i>IDH1</i> mutation and 1p/19q co-deletion	21	39	0.82 (0.48–1.42)	0.484
rLTL in non-GBM with <i>EGFR</i> amplification	13	25	1.18 (0.56–2.47)	0.659
rLTL in all GBM	199	387	1.16 (0.97–1.39)	0.097
rLTL in GBM with <i>IDH1</i> wildtype	190	370	1.11 (0.92–1.34)	0.256
rLTL in GBM with <i>IDH1</i> mutation	8	15	3.65 (1.14–11.72)	0.029
rLTL in GBM with <i>EGFR</i> amplification	87	167	1.11 (0.84–1.47)	0.485

were de-identified to remove any bias, and analyzed by a neuropathologist.

Fluorescent in situ hybridization (FISH)

Slides were deparaffinized in xylene and dehydrated in absolute alcohol. Slides were placed in a pre-treatment solution for 12 min at 80 °C and protease solution for 45 min at 37 °C (according to the instruction in Vysis pre-treatment kit IV (Abbot, Abbott Park, Illinois). Slides were washed in H₂O and dehydrated in a series of 70%, 80% and absolute ethanol. Each section was covered with Vysis LSI 1p36 / LSI 1q25 and LSI 19q13/19p13 dual-color probe (Abbott) for detection of chromosome 1p or 19q and Vysis *EGFR/CEP7* FISH probe Kit (Abbott) for detection *EGFR* amplification. The sections were co-denatured at 80 °C for 6 min and hybridized over night at 37 °C. Unspecific staining was removed using wash buffer one and two according to the instruction in the Vysis pre-treatment kit IV (Abbott). Sections were counterstained using DAPI-Antifade (Cytocell, Cambridge, UK). Slides were coded to remove any bias, and two independent observers analyzed each slide. Tumors with deletion in more than 12% of the cells for chromosome 1p and 20% of the cells for chromosome 19q were defined as co-deleted. A ratio between the locus specific *EGFR* probe and the control probe *CEP7* (*EGFR/CEP7*) was calculated were ratios equal to 1 was considered as normal, while more than 10% cells with a ratio between one and two was considered as chromosomal gain, and more than 10% cells with a ratio greater than two was considered as amplification.

Statistical analyses

Odds ratios (ORs), 95% confidence intervals (CIs), and *P* values for glioma risk were calculated using unconditional logistic regression models adjusted for age at diagnosis (cases)/enrollment (controls) and sex using rLTL as a continuous variable. The continuous rLTL variable was scaled to unit variance by dividing by its standard deviation, prior to statistical modeling to facilitate model interpretation. Linear regression analyses were conducted to examine the correlation between rLTL, IDH1 mutation, 1p/19q co-deletion, and EGFR amplification in glioma cases. All statistical analyses were performed using the SPSS Statistics 24.0 software (IBM), and *P* < 0.05 was considered statistically significant.

Results

Telomere length and glioma risk

Longer rLTL in peripheral blood was associated with increased risk of glioma (OR = 1.16; 95% CI 1.02–1.31, *P* = 0.025) (Fig. 1), and this association rely mainly among females (OR = 1.29; 95% CI 1.40–13.91, *P* = 0.011) compared to males (OR = 1.07; 95% CI 0.57–3.76, *P* = 0.429) (data not shown). Although not statistically significant, the association was in the same direction in all investigated glioma subgroups, with the exception of glioma with *IDH1* mutation and 1p/19q co-deletion (for all glioma with *IDH1* mutation and 1p/19q co-deletion OR = 0.92; 95% CI 0.55–1.56, and for non-GBM (glioma grade II-III) with

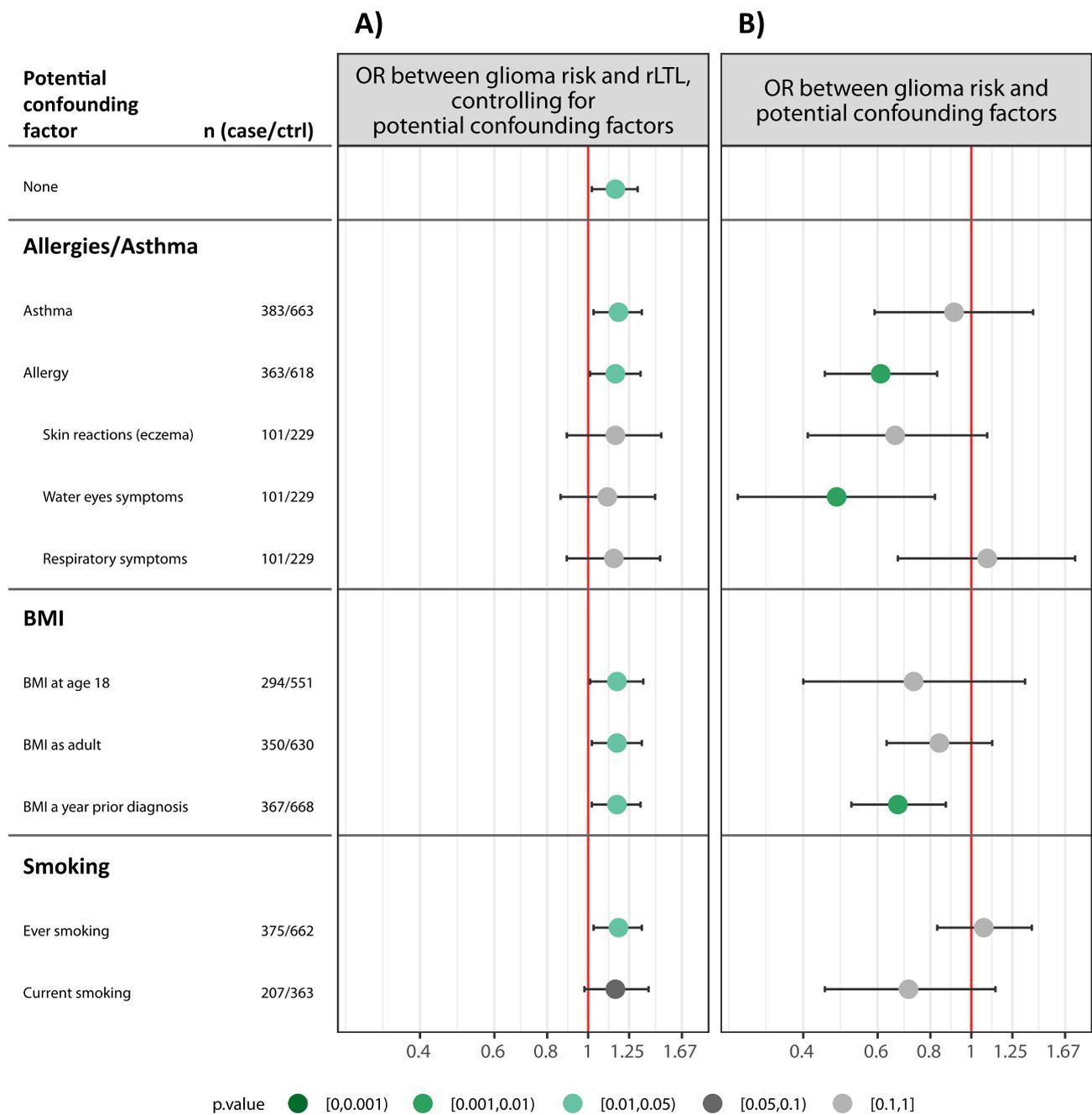


Fig. 1 a Forest plot illustrating the association between glioma risk and rLTL, controlling for the potential confounding factors; asthma (no asthma as reference), allergy (no allergy as reference), BMI categorized as underweight/normal versus overweight/obese (under-

weight/normal as reference), ever smoking (no smoking as reference), and current smoking (no smoking as reference). **b** Forest plot illustrating the association between glioma risk and potential confounding factors

IDH1 mutation and 1p/19q co-deletion OR = 0.82; 95% CI 0.48–1.42) (Table 2). rLTL for all controls and cases, and for women and men respectively are illustrated in Supplementary Fig. 1.

Telomere length and general health

Subjects with allergies had a lower glioma risk (OR = 0.61; 95% CI 0.45–0.83) (Fig. 1). We found no evidence that this association differed between high-grade and low-grade

glioma cases (data not shown). When analyzing specific types of allergy symptoms, watery eyes (OR = 0.48; 95% CI 0.28–0.82) was significantly protective against glioma, whereas we found no statistically significant association between glioma risk and respiratory symptoms (OR = 1.09; 95% CI 0.67–1.76) or eczema (OR = 0.66; 95% CI 0.41–1.09) (Fig. 1). Asthma was not significantly associated with glioma risk (OR = 0.91; 95% CI 0.59–1.40) (Fig. 1, Supplementary Fig. 2). High BMI a year prior diagnosis was significantly protective against glioma (OR = 0.67; 95% CI 0.52–0.87) (Fig. 1), and this association was not affected by the addition of rLTL to the model (data not shown). Smoking was not associated with glioma risk (Fig. 1). Adjusting for allergies, asthma, BMI, or smoking did not markedly change the association between rLTL and glioma risk (Fig. 1). Logistic regression models to investigate the interaction between rLTL and allergies, did not reveal any statistically significant effects on glioma risk ($P_{\text{interaction}} = 0.948$). rLTL for controls and cases, and the confounding factors are illustrated in Supplementary Figs. 2, 3 and 4. Among the controls in the Swedish cohort, we could not confirm previous studies showing shorter rLTL in individuals with allergic phenotypes (Supplementary Table 1).

Telomere length and tumor characteristics

Age at diagnosis and sex were the only factors associated with rLTL in linear regression analyses conducted to examine the association between rLTL and *IDH1* mutation, 1p/19q co-deletion as well as *EGFR* amplification in the tumor (Table 3). An association between *IDH1* mutation and

Discussion

In this study, rLTL was measured in cases and controls, and the interplay between rLTL, asthma and allergies, and glioma molecular phenotype was investigated. Telomere length in the hematopoietic compartment was used as a proxy for average individual telomere length in non-malignant tissues, to explore individual susceptibility to disease as telomere length is determined by both genetic [29] and environmental factors [30, 31].

Our results reveal that longer rLTL is associated with increased risk of glioma. Although not always statistically significant, the association was in the same direction in all glioma subgroups, with the exception of glioma with both *IDH1* mutation and 1p/19q co-deletion, suggesting that longer rLTL is not associated with risk of glioma with *IDH1* mutation and 1p/19q co-deletion (i.e., oligodendroglioma). Our finding of an association between longer rLTL and glioma risk confirms an earlier study investigating genotypically-estimated leukocyte telomere length (i.e., an estimation of telomere length based on a subject's genetic variants known to influence telomere length) and glioma risk [21]. Wang et al. [23] reported that both longer and shorter rLTL in peripheral blood are associated with higher risk of glioma, suggesting a U-shaped association between rLTL and glioma risk. On the other hand, Walcott et al. [22] reported no association overall, though found an association between short rLTL in peripheral blood and increased risk of glioma specifically among males. Contradictory to these reports, in this study we found a decreased risk of glioma in sub-

Table 3 Linear regression assessing associations between relative telomere length, age at diagnosis, sex, *IDH1* mutation and 1p/19q co-deletion separately or in combination, and *EGFR* amplification for glioma cases. Each variable was considered separately (univariate analysis) and in a model combining all variables (multivariate analysis)

	Univariate analysis		Multivariate analysis	
	Regression coefficient (SE)	<i>P</i> value	Regression coefficient (SE)	<i>P</i> value
Age at diagnosis	− 0.004 (0.001)	0.0001	− 0.003 (0.001)	0.0001
Sex ^a	− 0.046 (0.020)	0.021	− 0.052 (0.020)	0.010
<i>IDH1</i> mutation ^b	0.064 (0.027)	0.017	0.023 (0.039)	0.558
1p/19q co-deletion ^c	0.002 (0.029)	0.946	− 0.027 (0.040)	0.507
<i>IDH1</i> mutation and 1p/19q co-deletion ^d	0.011 (0.038)	0.768	− 0.019 (0.064)	0.771
<i>EGFR</i> amplification ^e	− 0.039 (0.021)	0.066	− 0.024 (0.022)	0.265
Adjusted <i>R</i> square			0.072	
<i>N</i>			298	

^aWomen used as reference category; ^b*IDH1* mutation used as reference category; ^c1p/19q co-deletion used as reference category; ^d*IDH1* mutation and 1p/19q co-deletion used as reference category; ^e*EGFR* amplification used as reference category; All categories are coded as 0 versus 1

rLTL was noted in the univariate analysis, but this interaction disappeared when including age at diagnosis and sex in the model ($P = 0.575$).

jects with short rLTL, specifically among females. Similarly, inconsistent results are not only restricted to glioma, since a number of studies have examined the relationship between telomere length measured in peripheral blood and various

cancer risk with conflicting results. The majority of the studies show that short telomere length are associated with increased risk of cancer [32–36], while the opposite, with an association between long telomere length and increased cancer risk, has been shown by other studies [37–40]. The biological mechanisms behind the association between altered telomere length in peripheral blood and increased cancer risk is unclear. It is also not evident whether alterations in telomere length in peripheral blood are caused by the cancer disease, or if the alterations are actually part of the cancer etiology. It is evident that the appearance of a malignant disease affects the whole individual, and the alterations in telomere length in peripheral blood seen at diagnosis might reflect the responses to the tumor. One limitation of this study is that the glioma patients were recruited at, or even after, their diagnosis. Consequently, blood samples from the patients were drawn at a time when medical treatment, stress, and inflammatory conditions may have had an effect on rLTL in peripheral blood. But, since stress and inflammatory conditions are expected to shorten telomere length in patients [41], these confounding factors would not affect the association between longer telomere length and glioma risk. In addition, our findings are supported by the previously mentioned study by Walsh et al. [21] in which telomere length was estimated based on genotypes (i.e., a Mendelian randomization approach), which would be independent of confounding factors such as medical treatment.

In concordance with previous studies, we report that individuals with allergies have a decreased risk of glioma [7, 42, 43] and allergy symptoms like watery eyes appeared to be more protective. In another GICC study by Amirian et al. 2016, where all the Swedish glioma cases- and controls were included, they reported that asthma and eczema were protective against glioma and that the association was significantly stronger in high-grade compared to low-grade glioma. Our data do not support the difference observed between high- and low-grade glioma, which might be explained by the fact that we analyzed a slightly smaller number of glioma cases- and controls from the Swedish cohort. In addition, although the association between glioma risk and eczema was not statistically significant in the present study, it was in the expected direction [7]. One hypothesis why allergy seems to be protective against glioma is that the reactive state of the immune system in atopic individuals may result in increased immune surveillance and limit abnormal cell proliferation, though the specific mechanism by how this could limit abnormal cell proliferation and thereby tumor growth remains to be clarified [44].

Shorter rLTL in peripheral blood has recently been proposed to be strongly associated with metabolic syndrome, especially in the female population [45]. Likewise, in a longitudinal study reported by Lee et al. [46], rLTL in

peripheral blood was shorter in an obese population. Our results propose that patients with a higher BMI one year prior diagnosis have decreased risk of glioma, whereas recently published data show that overweight or obesity are not connected to glioma risk [47]. The fact that this association was stronger for a high BMI one year prior to diagnosis, compared to the same association for BMI as adolescent or adult, raise the concern for reverse causality, as a low BMI may be coupled to undiagnosed disease. Although smoking has been suggested as a risk factor for glioma in some studies [48, 49], most studies conducted so far, including the present study [50–52], do not support an association between smoking and glioma risk.

IDH1 mutation, frequently co-occurring with molecular alterations including 1p/19q co-deletion, is an early event in the development of glial tumors and often accompanied by mutations influencing telomerase activity [53]. The tight linkage between *IDH1* mutations and mutations of genes and promoters involved in telomere regulation suggests that *IDH1* mutation may contribute to the genesis or selection of events that resolve telomeric dysfunction in the tumor, which may affect both immortalization and transformation [53]. Though, in this study, we did not find any association between rLTL measured in peripheral blood and *IDH1* mutation or other molecular alterations in the tumor.

A limitation with our study is that telomere length was measured in leukocytes and not in brain tissue. Although several studies have shown intra-individual synchrony in telomere length across somatic tissues in fast (e.g., blood and skin) as well as slowly proliferating tissues (e.g., fat, muscle, synovium), there is a lack of robust studies which have correlated leukocyte telomere length with normal and malignant brain tissue [54, 55]. Average rLTL may be influenced by immune response mediated telomerase activation, and TERT promoter mutations may influence telomere length in glioma tissue. Therefore, future studies measuring telomere length in glioma tissue will contribute to further understanding of the role of telomere biology in glioma pathology [56].

In conclusion, our study showed an association between long rLTL and risk of glioma. Even if previous studies have shown that asthma, BMI and smoking have an impact on telomere length, we were not able to establish any interactions of these risk factors in our cohort. Our study of measured rLTL support recent studies that associates high genetically estimated telomere length scores with increased glioma risk [57, 58]. This further indicates that telomere biology is involved in the complex pathways of gliomagenesis.

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