J Neurooncol (2011) 105:639–646
DOI 10.1007/s11060-011-0634-1

CLINICAL STUDY – PATIENT STUDY

A functional polymorphism in the *pre-miR-146a* gene is associated with risk and prognosis in adult glioma

Jennifer Permuth-Wey · Reid C. Thompson · L. Burton Nabors · Jeffrey J. Olson · James E. Browning · Melissa H. Madden · Y. Ann Chen · Kathleen M. Egan

Received: 22 February 2011 / Accepted: 17 June 2011 / Published online: 9 July 2011

Abstract MicroRNAs (miRNAs) are non-coding RNAs that function as post-transcriptional regulators of tumor suppressors and oncogenes. Single nucleotide polymorphisms (SNPs) in miRNAs may contribute to carcinogenesis by altering expression of miRNAs and their targets. A G>C polymorphism (rs2910164) in the *miR-146a* precursor sequence leads to a functional change associated with the risk for numerous malignancies. A role for this SNP in glioma pathogenesis has not yet been examined. We investigated whether rs2910164 genotypes influence glioma risk and prognosis in a multi-center case–control study comprised of 593 Caucasian glioma cases and 614 community-based controls. Unconditional logistic regression was used to estimate odds ratios (OR) and 95% confidence intervals (CI) for rs2910164 genotypes according to case status. Cox proportional hazards regression modeling was used to estimate hazards ratios (HR) and 95% CIs according to genotype among glioblastomas, the most lethal glioma subtype. An increased glioma risk was observed among rs2910164 minor allele (C) carriers (per allele OR (95% CI) = 1.22 (1.01–1.46, $p_{trend} = 0.039$)). The association was stronger among older subjects carrying at least one copy of the C allele (OR (95% CI) = 1.38 (1.04–1.83, $P = 0.026$). Mortality was increased among minor allele carriers (HR (95% CI) = 1.33 (1.03–1.72, $P = 0.029$)), with the association largely restricted to females (HR (95% CI) = 2.02 (1.28–3.17, $P = 0.002$)). We provide novel data suggesting rs2910164 genotype may contribute to glioma susceptibility and outcome. Future studies are warranted to replicate these findings and characterize mechanisms underlying these associations.

Keywords Genotype · Glioma · Susceptibility · Single nucleotide polymorphism · MicroRNA

J. Permuth-Wey · J. E. Browning · M. H. Madden · K. M. Egan (✉)
Department of Cancer Epidemiology, Division of Population Sciences, H. Lee Moffitt Cancer Center and Research Institute, 12902 Magnolia Drive, MRC-CANCONT, Tampa, FL 33612-9416, USA
e-mail: Kathleen.egan@moffitt.org

R. C. Thompson
Department of Neurological Surgery, Vanderbilt University Medical Center, Nashville, TN 37232, USA

L. Burton Nabors
Neuro-oncology Program, University of Alabama at Birmingham, Birmingham, AL 35294, USA

J. J. Olson
Department of Neurosurgery, Emory University School of Medicine, Atlanta, GA 30322, USA

Y. Ann Chen
Department of Biostatistics, H. Lee Moffitt Cancer Center & Research Institute, Tampa, FL 33612, USA

Introduction

Approximately 22,000 individuals are diagnosed with primary malignant brain tumors (BT) in the US each year, and about 13,000 people die from them [1]. Gliomas, the most common and deadly type of BT, derive from glial cells (astrocytes or oligodendrocytes) that surround and support neurons in the brain [2]. Although various environmental factors have been implicated in epidemiological studies of glioma, a high dose of ionizing radiation is the only well-established risk factor for the disease [3]. A role for genetic susceptibility has been highlighted by familial aggregation studies [4, 5] and rare mendelian cancer predisposition syndromes such as Neurofibromatosis, Li-Fraumeni

syndrome, and Turcot syndrome [3]. Additionally, candidate gene approaches have identified associations between glioma susceptibility and single nucleotide polymorphisms (SNPs) in genes affecting carcinogen metabolism, DNA repair, cell cycle regulation, and inflammation [6]. Furthermore, two glioma genome-wide association studies (GWAS) [7, 8] have concordantly identified glioma risk variants in *CDKN2B, RTEL1,* and *TERT,* and several other variants implicated in these GWAS have been validated in independent studies [9]. Despite these advances, the molecular mechanisms that contribute to gliomagenesis remain poorly understood.

Emerging evidence supports a role for microRNAs (miRNAs) in glioma development and/or progression. MiRNAs are a class of ~22-nucleotide-long non-protein coding RNAs that are thought to regulate up to a third of all protein-coding genes by binding to the 3′ untranslated region (UTR) of target gene messenger RNA (mRNA), causing translational repression and/or mRNA degradation [10]. MiRNAs have been implicated in various tumorigenic processes, including cell growth, differentiation, apoptosis, and metastasis [10]. miRNAs have been shown to regulate oncogenic pathways in gliomas, and aberrant miRNA expression has been consistently reported in glioma tumors as compared to normal tissue [11, 12].

Deregulated expression of miRNAs and their targets may occur as a consequence of functional polymorphisms in the miRNA sequence [13–17]. A G>C polymorphism (rs2910164) in the *miR-146a* precursor has been associated with early-onset familial breast or ovarian cancer [15], and the risk of papillary thyroid cancer [14], hepatocellular cancer [17], esophageal squamous cell cancer [18], gastric cancer [19, 20], and prostate cancer [16]. However, no study has examined the influence of the *miR-146a* variant in glioma. Based on a growing body of evidence regarding the functional role of *miR-146a* in tumorigenesis and other physiological processes (i.e., innate immune and inflammatory responses) linked to gliomagenesis [21–23], we hypothesized that *miR-146a* rs2910164 may be associated with glioma risk and/or prognosis. Herein we report results from our evaluation of rs2910164 in a large series of glioma cases and controls enrolled in the case–control Study of Glioma in the Southeast (*GliomaSE*), a multi-center, clinic-based case–control study conducted at medical centers in the Southeastern United States (US).

Methods

Study population

The study population has been described previously [9]. Briefly, cases included individuals aged 18 and above with a recent diagnosis (within 3 months) of histologically confirmed primary glioma who were identified in neurosurgery and neuro-oncology clinics at several major medical and oncology centers in the Southeastern US: Vanderbilt University Medical Center (Nashville, Tennessee); Moffitt Cancer Center and Research Institute (Tampa, Florida); the University of Alabama (Birmingham, AL); Emory University (Atlanta, GA), and the Kentuckiana Cancer Institute (Louisville, KY). Cancer-free controls were comprised of friends, in-laws and other associates of the cases or were sampled from the communities giving rise to the cases using white page listings. Controls reporting a personal history of brain tumor were excluded from analysis.

Eighty-seven percent of eligible glioma patients participated in the study and provided a DNA sample. Of those not included in the study, 7% refused (typically due to illness) and 6% died before they could be approached or a DNA sample could be collected after providing informed consent. Cases were enrolled a median of 1.0 month following the diagnosis of glioma. Among community controls, 49.6% of confirmed eligible households yielded a study participant.

The study was approved by investigational review committees at each participating center, and all subjects enrolled in the study provided written informed consent.

Biospecimen and data collection

Genomic DNA samples for genotyping were obtained by oral rinse or the saliva method (www.dnagenotek.com). DNA samples were sent to the core genotyping facility at Vanderbilt (during the pilot phase) or to the tissue core at Moffitt (the coordinating center) for DNA extraction and storage according to standard procedures. Demographic data and information on known and suspected glioma risk factors were collected through interviewer-administered questionnaires.

Genotyping method and quality control

Genotyping was performed at the Center for Genome Technology at the Hussman Institute for Human Genomics, University of Miami using Illumina's GoldenGate technology (Illumina, San Diego, CA). As described previously [9], a custom panel of 1536 SNPs was developed to include SNPs in genes linked to cancer in GWAS or large consortial studies, along with putatively functional SNPs (such as rs2910164) identified through a comprehensive literature review. Each 96-well plate contained 3 micrograms of DNA of random mixtures of 88 unique case and control samples, six blind duplicates, and two replicates of a CEPH family trio

(mother, father, child) from the Coriell Institute. Laboratory personnel were blinded as to the case–control status of the samples. Among 94 pairs of replicate samples, the mean concordance rate for this SNP was 99.8%.

Of 699 glioma cases and 704 controls submitted for genotyping, we excluded 33 cases (4.7%) and 31 controls (4.4%) with low call rates (below 75% for the entire SNP array), and an additional 27 cases (3.9%) and 24 controls (3.4%) who reported non-Caucasian parent(s). A further 46 cases and 35 controls had no genotype call for this variant, resulting in a total of 593 glioma cases and 614 controls (including 520 community- and 94 'friend' controls) included in the analysis.

Statistical analysis

Unconditional logistic regression was used to estimate OR and 95% CI for rs2910164 assuming a log-additive model; tests for trend were performed by including an ordinal term corresponding to the number of minor alleles (0, 1 or 2), and models were adjusted for age and gender. During modeling, the major allele (G) was considered to be the reference allele. Due to the heterogeneous nature of gliomas [3], multinomial logistic regression was used to evaluate rs2910164 genotype associations according to glioma histologic subtype, including glioblastomas (GBM), anaplastic and lower grade astrocytomas, and oligodendroglial tumors (oligodendrogliomas and mixed oligoastrocytomas).

Cox proportional hazards regression was used to assess whether rs2910164 genotypes were associated with mortality rates among the GBM cases. Hazard ratios (HR) and 95% CIs were estimated per allele and for heterozygotes and minor allele homozygotes combined (GC+CC) compared with major allele homozygotes (GG) as the referent group, with adjustment for age and gender. Associations were examined among all GBM patients ($N = 329$), and among those treated with the current standard of care in GBM ($N = 245$) e.g., surgical resection followed by radiation and temozolomide chemotherapy.

For both the association and survival analyses, stratification was conducted to examine associations according to age- and gender. A P-value <0.05 was considered statistically significant, and all statistical tests were two-sided. Statistical analysis was performed using SAS Version 9.1 (SAS Institute, Inc., Cary, NC). Kaplan–Meier curves were generated using R version 2.10.1.

Results

Selected characteristics of the study population are displayed in Table 1. The median age at enrollment was 55 years (range: 19–89) in cases and 58 years (range: 19–89) in controls. Males comprised 61% of cases and 58% of controls. Most subjects resided in Tennessee (30%) or Florida (25%), whereas a fewer number resided in Alabama (15%), Kentucky (13%), Georgia (11%), or another state (6%). The case group was comprised primarily of GBM ($n = 329$, 51%), lower-grade astrocytic tumors ($n = 145$, 23%), and oligodendroglial tumors ($n = 89$, 14%) (Table 1).

Table 1 Selected characteristics of glioma cases and controls

Variable	Cases ($N = 593$)	Controls ($N = 614$)
Age, median (range)	55 (19–89)	58 (19–89)
Gender, N (%)		
Male	364 (61)	355 (58)
Female	229 (39)	259 (42)
State of residence, N (%)		
TN	155 (27)	203 (34)
FL	157 (27)	141 (24)
AL	99 (17)	80 (13)
KY	78 (14)	85 (14)
GA	68 (12)	65 (11)
Other	20 (3)	26 (4)
Histological type, N (%)		
Glioblastomas[a]	329 (51)	–
Lower grade astrocytomas[b]	145 (23)	–
Oligodendroglial tumors[c]	89 (14)	–
Other gliomas[d]	30 (5)	–

Unequal column totals indicate missing data

[a] ICD-0 code 9440/3

[b] Includes 76 grade 3 anaplastic astrocytomas (ICD-0 9401/3) and 69 grade 1 ($N = 4$) or 2 ($N = 65$) astrocytomas (ICD-0 9384/1, 9421/1, 9400/3, 9424/3)

[c] Includes pure oligodendrogliomas (ICD-0 9450/3, 9451/3) and mixed oligodendroglial and astrocytic tumors (ICD-0 9383/3)

[d] Includes rare glioma variants and gliomas with unspecified histology and/or grade

We first examined allele and genotype frequencies for the rs2910164 G>C polymorphism in our study population. The frequency of the minor allele (C) among cases and controls was 25 and 22%, respectively (Table 2). Adjustment for age and gender did not change parameter estimates or confidence intervals appreciably (data not shown), and results from unadjusted models are presented. We observed an increased risk of glioma, overall, among rs2910164 minor allele (C) carriers (per allele OR (95% CI) = 1.22 (1.01–1.46, $p_{trend} = 0.039$)) (Table 2). When we evaluated the rs2910164 genotype according to grade and histological subtype of astrocytic glioma, a statistically significant positive association was observed for lower

Table 2 Associations between rs2910164 and glioma risk according to astrocytic histologic subtype and grade

	All glioma subtypes[a] (N = 593 cases, 614 controls)				Lower grade astrocytic tumors (N = 145 cases, 614 controls)				Glioblastomas (N = 329 cases, 614 controls)			
	Cases N (%)	Controls[b] N (%)	OR (95% CI)	P	Cases N (%)	Controls N (%)	OR (95% CI)	P	Cases N (%)	Controls N (%)	OR (95% CI)	P
Allele												
G	888 (75)	964 (79)	Ref.	–	210 (72)	964 (79)	Ref.	–	493 (75)	964 (79)	Ref.	–
C	298 (25)	264 (22)	1.23 (1.01, 1.48)	**0.035**	80 (28)	264 (22)	1.39 (1.04, 1.86)	**0.026**	165 (25)	264 (22)	1.22 (0.98, 1.53)	0.077
Genotype												
GG	345 (58)	375 (61)	Ref.	–	78 (54)	375 (61)	Ref.	–	194 (58)	375 (61)	Ref.	–
CG	198 (33)	214 (35)	1.01 (0.79, 1.28)	0.963	54 (37)	214 (35)	1.21 (0.83, 1.78)	0.326	105 (32)	214 (35)	0.95 (0.71, 1.27)	0.721
CC	50 (8)	25 (4)	2.17 (1.32, 3.59)	**0.002**	13 (9)	25 (4)	2.50 (1.23, 5.10)	**0.012**	30 (9)	25 (4)	2.32 (1.33, 4.05)	**0.003**
Per allele[c]			1.22 (1.01, 1.46)	**0.039**			1.40 (1.04, 1.87)	**0.026**			1.21 (0.97, 1.51)	0.083

Statistically significant P values ($P < 0.05$) are in bold type

Some percentages do not add up to 100 due to rounding

OR odds ratio; *CI* confidence interval

[a] Includes lower grade astrocytic tumors, glioblastomas, and oligodendroglial tumors

[b] The genotype frequencies among the control subjects were in agreement with Hardy–Weinberg equilibrium ($P = 0.62$)

[c] OR and 95% CI per copy of the minor allele (C) using a log-additive model; corresponding P-value represents p-trend

grade astrocytomas (per allele OR (95% CI) = 1.40 (1.04–1.87, $p_{trend} = 0.026$), and a nonsignificant positive association was observed for GBMs (per allele OR (95% CI) = 1.21 (0.97–1.51, $p_{trend} = 0.083$)) (Table 2). For both subtypes, the data were most consistent with a recessive model: those carrying 2 at-risk C alleles had a 2.4-times higher risk of astrocytic glioma, combining GBM and lower grade astrocytic tumors (OR 2.37; 95% CI 1.41–3.98; $P = 0.001$) when compared to those with the GG genotype (data not shown). In contrast, we observed no association among the 89 oligodendroglial tumors (per allele OR (95% CI) = 0.92 (0.62–1.37, $p_{trend} = 0.696$) (data not shown), though statistical power was limited in these analyses.

We next examined the association between rs2910164 genotype and mortality among the 329 GBM cases. Among these patients, two hundred forty-four patients (74%) died from GBM a median of 11.5 months (range: 0.8–58.8 months) following diagnosis. Among 85 surviving patients, the median follow-up time was 18.5 months (range: 3.1–50.8 months) (data not shown). Significantly decreased survival rates were observed among individuals having at least one copy of the minor allele (HR (95% CI) = 1.33 (1.03–1.72, $P = 0.029$)) compared to those with the GG genotype (Table 3), with the association driven by the excess mortality among heterozygotes (HR (95% CI) = 1.44 (1.10–1.88, $P = 0.009$)). Figure 1 shows genotype-specific Kaplan–Meier survival probabilities by month post GBM diagnosis. Individuals with the GC genotype had increased mortality whereas those homozygous for the major G allele had consistently decreased mortality when compared to those with other genotypes (log rank test P value = 0.023). Hazards ratios were similar among the subgroup of patients treated with the standard of care in GBM ($N = 245$), though associations were nonsignificant due to diminished power in this subset of patients (data not shown).

Table 3 Hazards ratios between rs2910164 genotype and glioblastoma survival

	Glioblastomas ($N = 329$)		
	N	HR (95% CI)[a]	P
Genotype			
GG	194	Ref.	–
CG	105	1.44 (1.10, 1.88)	**0.009**
CC	30	0.99 (0.61, 1.60)	0.965
Per Allele[b]		1.14 (0.94, 1.37)	0.172
CG/CC[c]	135	1.33 (1.03, 1.72)	**0.029**

Statistically significant P values ($P < 0.05$) are in bold type

Some percentages do not add up to 100 due to rounding

[a] Unadjusted hazards ratios (HR) and 95% Confidence Intervals (CI)

[b] HR and 95% CI per copy of the minor allele (C) using a log-additive model; corresponding P-value represents p-trend

[c] CG/CC genotypes versus the reference GG genotype

In exploratory analyses, we considered associations according to age (using the median age of 58 years in the controls as the cut-point) and gender (Table 4). The rs2910164 at-risk C allele was significantly associated with glioma risk among older (OR (95% CI) = 1.38 (1.04–1.83, $P = 0.026$)) though not younger (OR (95% CI) = 1.16

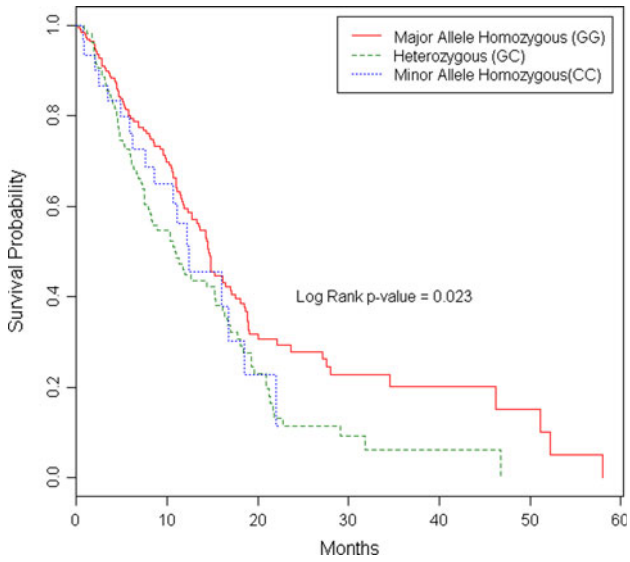

Fig. 1 Kaplan–Meier survival curves among GBM cases stratified by *pre-miR 146a* rs2910164 genotype. Survival time is defined as the time from GBM diagnosis to death or last follow-up among surviving cases. The log-rank method was used to test for genotype-specific differences in survival distributions. The *solid line* represents cases with wild-type GG genotype, *dashes* represent the heterozygous GC genotype, and *dots* represent cases carrying two minor/variant CC alleles

(0.88–1.52, $P = 0.284$)) persons. Odds ratios were of a similar magnitude in males and females, although results did not reach statistical significance in either sub-group. The rs2910164 C allele was significantly associated with poorer survival among younger (HR (95% CI) = 1.59 (1.03–2.47, $P = 0.037$)) but not older persons (HR (95% CI) = 1.19 (0.87–1.63, $P = 0.283$)), and increased mortality in C allele carriers was observed in females (HR (95% CI) = 2.02 (1.28–3.17, $P = 0.002$)) but not in males (HR (95% CI) = 1.02 (0.75–1.40, $P = 0.895$)) (Table 4).

Discussion

In the current study, we evaluated the association between a functional polymorphism in *miR-146a* (rs2910164) and glioma risk and prognosis in a large population of 593 incident pathologically confirmed Caucasian glioma cases and 614 controls. Our findings suggest that the rs2910164 CC/GC genotypes are associated with an increased risk of glioma, particularly among older persons. Furthermore, the C allele was associated with poorer survival among patients with glioblastoma, an association limited to females in the present data.

An association between rs2910164 *miR-146a* genotype and cancer risk has been reported for several malignancies, although results are not consistent. Jazdewski et al. [14] reported that the GC genotype was associated with an increased risk for papillary thyroid cancer, whereas both homozygous genotypes were protective. Xu. et al. [16] found that the CC genotype was associated with a decreased prostate cancer risk compared to the GC/CC genotypes. In a study of familial breast and ovarian cancer patients, Shen et al. [15] reported that carriers of the C allele were susceptible to an earlier age at disease onset. Of case–control studies that considered CC as the reference genotype, Xu et al. [17] found that the GG genotype was associated with increased risk for hepatocellular carcinomas among males, and Guo et al. [18] showed that GG genotype was associated with increased esophageal squamous cell carcinoma risk, especially among smokers. A Chinese case–control study of gastric cancer found that compared to the CC genotype, the GG and GC genotypes were associated with an increased gastric cancer risk in young individuals, non-smokers, and males [19], while a Japanese study of gastric cancer that used the GG genotype as the reference found that C allele carriers were at increased risk [20]. Inconsistent results across studies may represent differences in tumor-specific etiology,

Table 4 Stratified analysis of rs2910164 (G>C) genotype on overall glioma risk and glioblastoma survival

Variable	Glioma risk[a] ($N = 474$ cases, 614 controls)		GBM survival ($N = 329$ GBM cases)	
	CG/CC vs. GG OR (95% CI)	*P* value	CG/CC vs. GG HR (95% CI)	*P* value
Age[b]				
<58	1.16 (0.88, 1.52)	0.284	1.59 (1.03, 2.47)	**0.037**
≥58	1.38 (1.04, 1.83)	**0.026**	1.19 (0.87, 1.63)	0.283
Gender				
Male	1.23 (0.95, 1.59)	0.116	1.02 (0.75, 1.40)	0.895
Female	1.32 (0.97, 1.78)	0.076	2.02 (1.28, 3.17)	**0.002**

Statistically significant *P* values ($P < 0.05$) are in bold type

OR = odds ratio; *CI* = confidence interval; *HR* = hazards ratio; *GBM* = glioblastoma

[a] Analyses restricted to astrocytic tumors (i.e., lower grade astrocytic tumors and GBM)

[b] Stratified according to the median age in controls (i.e. 58 years)

differences in the ethnicity of study populations, and use of different reference alleles.

In the present study, rs2910164 genotypes were significantly associated with glioma risk among older persons (above the median age in the controls of 58 years), but not among younger persons, consistent with other studies that also showed age-specific effects for this SNP [15, 19]. These findings, if not due to chance, are consistent with the possibility that expression of the genotype is conditional on a lifetime of accumulated environmental carcinogens or potentially a weakening immune system in older individuals. Survival analyses according to the rs2910164 genotypes suggested an increased mortality in carriers of the variant C allele, a finding largely restricted to females. Although chance may account for these findings, such a result may potentially indicate that expression of the risk-associated variant depends on the hormonal mileau [24].

The rs2910164 SNP involves a G:U to C:U mispairing in the hairpin of the *miR146a* precursor. This mismatch has been shown to lead to altered processing and expression of the mature miRNA sequence. One report involving breast cancer cell-lines [15] found that the C-allelic precursor associated with early onset breast cancer displayed increased mature *miR-146a* production compared with the G-allelic precursor, whereas three other reports involving papillary thyroid cancer [14], prostate cancer [16], and hepatocellular cancer cells [17] indicated an association of the C allele with decreased mature *miR-146a* expression. Loss-of-function of *miR-146a* has been shown to promote cancer cell migration, invasion, and metastasis in vitro in prostate cells [25]. In the present report, the rs2910164 C allele conferred both increased risk and poorer survival, suggesting that this variant may promote loss-of-function of *miR-146a* and contribute to subsequent glioma risk and invasion. Although further replication in a larger sample size is warranted, our findings suggest that these associations may be limited to astrocytic tumors (i.e., low-grade astrocytomas and GBMs) rather than oligodendroglial tumors. Given this observation, it could be speculated that the mechanism underlying these associations may be more likely to involve expression of mesenchymal genes as opposed to proneural genes that have been linked to oligodendroglial tumors [26].

It has been proposed that rs2910164 genotype contributes to carcinogenesis by mediating interactions with key target genes [14]. As Jazdewski et al. [14] demonstrated, the C allele of rs2910164 may affect target mRNA binding by conferring less efficient inhibition of target genes. *miR-146a* is an nuclear factor-kappa beta (*NF-kβ*)-dependent gene that is known to target tumor necrosis receptor-associated factor 6 (*TRAF6*) and interleukin-1 receptor-associated kinase 1 (*IRAK1*), key adaptor molecules downstream of the Toll-like and cytokine receptors that are involved in cell growth and immune recognition [27]. Although specific studies of these target molecules in gliomas are lacking, it is well-established that these gene families [28] and the *NF-kβ*-pathway [29] play a role in driving the glioblastoma tumor phenotype. Thus, rs2910164 may contribute to gliomagenesis by disrupting *miR-146a* as a mediator of the pro-apoptotic transcription factor NF-*κβ* [30]. Another validated target of *miR-146a* is the mesenchymal marker *CXCR4* (chemokine C-X-C motif receptor 4) [31], a *CXCL12/SDF-1* chemokine receptor involved in homing/mobilization of stem cells. Down-modulation of *mir-146a* was shown to cause an increase in translation of target *CXCR4* mRNA in leukemic cells [31]. *CXCR4* has been shown to be over-expressed in GBM [32, 33], and its activation has been shown to promote motility, invasion, and proliferation of glioma cells [34–36]. Therefore, it is plausible that predisposition to glioma and/or glioma invasion in rs2910164 C allele carriers may develop through up-regulation of *CXCR4*, an area requiring further research.

Strengths of the current study include the relatively large sample size considering the rarity of gliomas, pathologic confirmation of all cases, the rapid case ascertainment which minimized the potential influence of survival bias, and the high quality genotype data, as evidenced by successful demonstration in these data [9] of risk associations with GWAS-identified SNPs in *TERT*, *RTEL1* and *CDKN2B* [7, 8], all associated with relatively modest relative risks (per-allele OR ranging from 1.15 to 1.39). A limitation of the present data is that we could not evaluate associations according to race, as most ($\sim$98%) subjects in the study are Caucasian. We are aware of only one case–control study in a population of Chinese individuals by Dou et al. [37] that has evaluated the association between glioma risk and a T>C polymorphism (rs11614913) in the *miR-196a* precursor that has been associated with decreased lung cancer survival and increased breast cancer risk [38, 39]. Dou et al. [37] found that the CC genotype of rs11614913 was associated with significantly decreased glioma risk (OR 0.74), with a stronger association observed in males (OR 0.69) and in patients with GBM (OR 0.58). Taken together, these findings suggest that evaluation of additional variants that may impact miRNA primary transcripts or miRNA-target interactions may be fruitful avenues for future research in glioma.

In summary, these results suggest for the first time that rs2910164 may contribute to glioma susceptibility and prognosis. Future studies are warranted to replicate these findings in a larger, independent population that is well-powered to examine potential heterogeneity according to histological subtypes of glioma. Furthermore, mechanistic studies are needed to further characterize rs2910164 genotype and its influence on *miR-146a* and target mRNA

expression in gliomas. Knowledge of inherited variation in miRNA-related genes may help to identify high-risk populations and aid in the development of diagnostic, prognostic, and therapeutic strategies to reduce the burden of gliomas and other malignancies.

Acknowledgments The authors wish to acknowledge the study participants without whom the research would not have been possible. We further wish to thank the clinicians and research staffs at participating medical centers for their contributions. Finally, we thank Ms. Anna Konidari and staff at the Center for Genome Technology at the Hussman Institute for Human Genomics, University of Miami for their expert technical assistance in genotyping. The project was supported by the National Institutes of Health (CA R01CA116174) and institutional funding provided by the Moffitt Cancer Center (Tampa, FL) and the Vanderbilt-Ingram Comprehensive Cancer Center (Nashville, TN).

References

1. American Cancer Society (2010) Cancer Facts and Figures 2010. American Cancer Society, Atlanta
2. Louis DN, Ohgaki H, Wiestler OD, Cavenee WK, Burger PC, Jouvet A, Scheithauer BW, Kleihues P (2007) The 2007 WHO classification of tumours of the central nervous system. Acta Neuropathol 114:97–109
3. Bondy ML, Scheurer ME, Malmer B, Barnholtz-Sloan JS, Davis FG, Il'yasova D, Kruchko C, McCarthy BJ, Rajaraman P, Schwartzbaum JA, Sadetzki S, Schlehofer B, Tihan T, Wiemels JL, Wrensch M, Buffler PA (2008) Brain tumor epidemiology: consensus from the brain tumor epidemiology consortium. Cancer 113:1953–1968
4. Hemminki K, Li X (2003) Familial risks in nervous system tumors. Cancer Epidemiol Biomarkers Prev 12:1137–1142
5. Scheurer ME, Etzel CJ, Liu M, El-Zein R, Airewele GE, Malmer B, Aldape KD, Weinberg JS, Yung WK, Bondy ML (2007) Aggregation of cancer in first-degree relatives of patients with glioma. Cancer Epidemiol Biomarkers Prev 16:2491–2495
6. Gu J, Liu Y, Kyritsis AP, Bondy ML (2009) Molecular epidemiology of primary brain tumors. Neurotherapeutics 6:427–435
7. Shete S, Hosking FJ, Robertson LB, Dobbins SE, Sanson M, Malmer B, Simon M, Marie Y, Boisselier B, Delattre JY, Hoang-Xuan K, El Hallani S, Idbaih A, Zelenika D, Andersson U, Henriksson R, Bergenheim AT, Feychting M, Lonn S, Ahlbom A, Schramm J, Linnebank M, Hemminki K, Kumar R, Hepworth SJ, Price A, Armstrong G, Liu Y, Gu X, Yu R, Lau C, Schoemaker M, Muir K, Swerdlow A, Lathrop M, Bondy M, Houlston RS (2009) Genome-wide association study identifies five susceptibility loci for glioma. Nat Genet 41:899–904
8. Wrensch M, Jenkins RB, Chang JS, Yeh RF, Xiao Y, Decker PA, Ballman KV, Berger M, Buckner JC, Chang S, Giannini C, Halder C, Kollmeyer TM, Kosel ML, LaChance DH, McCoy L, O'Neill BP, Patoka J, Pico AR, Prados M, Quesenberry C, Rice T, Rynearson AL, Smirnov I, Tihan T, Wiemels J, Yang P, Wiencke JK (2009) Variants in the CDKN2B and RTEL1 regions are associated with high-grade glioma susceptibility. Nat Genet 41:905–908
9. Egan KM, Thompson RC, Nabors LB, Olson JJ, Brat DJ, Larocca RV, Brem S, Moots PL, Madden MH, Browning JE, Ann Chen Y (2011) Cancer susceptibility variants and the risk of adult glioma in a US case-control study. J Neurooncol 1–8
10. Calin GA, Croce CM (2006) MicroRNA signatures in human cancers. Nat Rev Cancer 6:857–866
11. Silber J, James CD, Hodgson JG (2009) microRNAs in gliomas: small regulators of a big problem. Neuromolecular Med 11:208–222
12. Turner JD, Williamson R, Almefty KK, Nakaji P, Porter R, Tse V, Kalani MY (2010) The many roles of microRNAs in brain tumor biology. Neurosurg Focus 28: E3
13. Duan R, Pak C, Jin P (2007) Single nucleotide polymorphism associated with mature miR-125a alters the processing of pri-miRNA. Hum Mol Genet 16:1124–1131
14. Jazdzewski K, Murray EL, Franssila K, Jarzab B, Schoenberg DR, de la Chapelle A (2008) Common SNP in pre-miR-146a decreases mature miR expression and predisposes to papillary thyroid carcinoma. Proc Natl Acad Sci USA 105:7269–7274
15. Shen J, Ambrosone CB, DiCioccio RA, Odunsi K, Lele SB, Zhao H (2008) A functional polymorphism in the miR-146a gene and age of familial breast/ovarian cancer diagnosis. Carcinogenesis 29:1963–1966
16. Xu B, Feng NH, Li PC, Tao J, Wu D, Zhang ZD, Tong N, Wang JF, Song NH, Zhang W, Hua LX, Wu HF (2010) A functional polymorphism in Pre-miR-146a gene is associated with prostate cancer risk and mature miR-146a expression in vivo. Prostate 70:467–472
17. Xu T, Zhu Y, Wei QK, Yuan Y, Zhou F, Ge YY, Yang JR, Su H, Zhuang SM (2008) A functional polymorphism in the miR-146a gene is associated with the risk for hepatocellular carcinoma. Carcinogenesis 29:2126–2131
18. Guo H, Wang K, Xiong G, Hu H, Wang D, Xu X, Guan X, Yang K, Bai Y (2010) A functional varient in microRNA-146a is associated with risk of esophageal squamous cell carcinoma in Chinese Han. Fam Cancer
19. Zeng Y, Sun QM, Liu NN, Dong GH, Chen J, Yang L, Wang B (2010) Correlation between pre-miR-146a C/G polymorphism and gastric cancer risk in Chinese population. World J Gastroenterol 16:3578–3583
20. Okubo M, Tahara T, Shibata T, Yamashita H, Nakamura M, Yoshioka D, Yonemura J, Kamiya Y, Ishizuka T, Nakagawa Y, Nagasaka M, Iwata M, Yamada H, Hirata I, Arisawa T (2010) Association study of common genetic variants in pre-microRNAs in patients with ulcerative colitis. J Clin Immunol 31(1):69–73
21. Li L, Chen XP, Li YJ (2010) MicroRNA-146a and human disease. Scand J Immunol 71:227–231
22. Barcellos-Hoff MH, Newcomb EW, Zagzag D, Narayana A (2009) Therapeutic targets in malignant glioblastoma microenvironment. Semin Radiat Oncol 19:163–170
23. Zhou M, Wiemels JL, Bracci PM, Wrensch MR, McCoy LS, Rice T, Sison JD, Patoka JS, Wiencke JK (2010) Circulating levels of the innate and humoral immune regulators CD14 and CD23 are associated with adult glioma. Cancer Res 70:7534–7542
24. Kabat GC, Etgen AM, Rohan TE (2010) Do steroid hormones play a role in the etiology of glioma? Cancer Epidemiol Biomarkers Prev 19:2421–2427
25. Lin SL, Chiang A, Chang D, Ying SY (2008) Loss of mir-146a function in hormone-refractory prostate cancer. RNA 14:417–424
26. Verhaak RG, Hoadley KA, Purdom E, Wang V, Qi Y, Wilkerson MD, Miller CR, Ding L, Golub T, Mesirov JP, Alexe G, Lawrence M, O'Kelly M, Tamayo P, Weir BA, Gabriel S, Winckler W, Gupta S, Jakkula L, Feiler HS, Hodgson JG, James CD, Sarkaria JN, Brennan C, Kahn A, Spellman PT, Wilson RK, Speed TP, Gray JW, Meyerson M, Getz G, Perou CM, Hayes DN (2010) Integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFRA, IDH1, EGFR, and NF1. Cancer Cell 17:98–110
27. Taganov KD, Boldin MP, Chang KJ, Baltimore D (2006) NF-kappaB-dependent induction of microRNA miR-146, an inhibitor targeted to signaling proteins of innate immune responses. Proc Natl Acad Sci USA 103:12481–12486

28. Kargiotis O, Rao JS, Kyritsis AP (2006) Mechanisms of angiogenesis in gliomas. J Neurooncol 78:281–293
29. Atkinson GP, Nozell SE, Benveniste ET (2010) NF-kappaB and STAT3 signaling in glioma: targets for future therapies. Expert Rev Neurother 10:575–586
30. Jazdzewski K, Liyanarachchi S, Swierniak M, Pachucki J, Ringel MD, Jarzab B, de la Chapelle A (2009) Polymorphic mature microRNAs from passenger strand of pre-miR-146a contribute to thyroid cancer. Proc Natl Acad Sci USA 106:1502–1505
31. Labbaye C, Spinello I, Quaranta MT, Pelosi E, Pasquini L, Petrucci E, Biffoni M, Nuzzolo ER, Billi M, Foa R, Brunetti E, Grignani F, Testa U, Peschle C (2008) A three-step pathway comprising PLZF/miR-146a/CXCR4 controls megakaryopoiesis. Nat Cell Biol 10:788–801
32. Zagzag D, Lukyanov Y, Lan L, Ali MA, Esencay M, Mendez O, Yee H, Voura EB, Newcomb EW (2006) Hypoxia-inducible factor 1 and VEGF upregulate CXCR4 in glioblastoma: implications for angiogenesis and glioma cell invasion. Lab Invest 86:1221–1232
33. Sehgal A, Keener C, Boynton AL, Warrick J, Murphy GP (1998) CXCR-4, a chemokine receptor, is overexpressed in and required for proliferation of glioblastoma tumor cells. J Surg Oncol 69:99–104
34. do Carmo A, Patricio I, Cruz MT, Carvalheiro H, Oliveira CR, Lopes MC (2010) CXCL12/CXCR4 promotes motility and proliferation of glioma cells. Cancer Biol Ther 9:56–65
35. Esencay M, Newcomb EW, Zagzag D (2010) HGF upregulates CXCR4 expression in gliomas via NF-kappaB: implications for glioma cell migration. J Neurooncol 99:33–40
36. Ehtesham M, Winston JA, Kabos P, Thompson RC (2006) CXCR4 expression mediates glioma cell invasiveness. Oncogene 25:2801–2806
37. Dou T, Wu Q, Chen X, Ribas J, Ni X, Tang C, Huang F, Zhou L, Lu D (2010) A polymorphism of microRNA196a genome region was associated with decreased risk of glioma in Chinese population. J Cancer Res Clin Oncol 136(12):1853–1859
38. Hu Z, Chen J, Tian T, Zhou X, Gu H, Xu L, Zeng Y, Miao R, Jin G, Ma H, Chen Y, Shen H (2008) Genetic variants of miRNA sequences and non-small cell lung cancer survival. J Clin Invest 118:2600–2608
39. Hu Z, Liang J, Wang Z, Tian T, Zhou X, Chen J, Miao R, Wang Y, Wang X, Shen H (2009) Common genetic variants in pre-microRNAs were associated with increased risk of breast cancer in Chinese women. Hum Mutat 30:79–84