Cellular and Molecular Neurobiology (2022) 42:1949–1964
https://doi.org/10.1007/s10571-021-01075-6

ORIGINAL RESEARCH

The Influence of *NDRG1* Single Nucleotide Polymorphisms on Glioma Risk and Prognosis in Chinese Han Population

Yonghui Yang[1] · Yimin Cheng[2,3] · Liting Yang[2] · Guangyao Shi[2] · Peng Chen[2] · Liang Li[4] · Hangrong Fang[5] · Chao Chen[2] · Tianbo Jin[6,7]

Received: 12 November 2020 / Accepted: 1 March 2021 / Published online: 12 March 2021

Abstract

Glioma is a highly fatal malignant tumor with a high recurrence rate, poor clinical treatment effect, and prognosis. We aimed to determine the association between single nucleotide polymorphisms (SNPs) of *NDRG1* and glioma risk and prognosis in the Chinese Han population. 5 candidate SNPs were genotyped by Agena MassARRAY in 558 cases and 503 controls; logistic regression was used to analyze the relationship between SNPs and glioma risk. We used multi-factor dimensionality reduction to analyze the interaction of 'SNP–SNP'; the prognosis analysis was performed by log-rank test, Kaplan–Meier analysis, and Cox regression model. Our results showed that the polymorphisms of rs3808599 was associated with the reduction of glioma risk in all participants (OR 0.41, $p=0.024$) and the participants ≤ 40 years old (OR 0.30, $p=0.020$). rs3802251 may reduce glioma risk in all participants (OR 0.79, $p=0.008$), the male participants (OR 0.68, $p=0.033$), and astrocytoma patients (OR 0.81, $p=0.023$). rs3779941 was associated with poor glioma prognosis in all participants (HR = 2.59, $p=0.039$) or astrocytoma patients (HR = 2.63, $p=0.038$). We also found that the key factors for glioma prognosis may include surgical operation, radiotherapy, and chemotherapy. This study is the first to find that *NDRG1* gene polymorphisms may have a certain association with glioma risk or prognosis in the Chinese Han population.

Keywords Glioma · *NDRG1* · Single nucleotide polymorphisms · Case–control

Introduction

Glioma is a tumor that originates from neuroectodermal mesenchymal cells and accounts for about 40–50% of brain tumors. It is the most common intracranial malignant tumor (McNeill 2016). According to the 2016 World Health Organization glioma classification standard, gliomas mainly include astrocytoma, oligodendrocytoma, oligoastrocytoma, et al. (Gupta and Dwivedi 2017). Glioma has a high recurrence rate and high mortality. And in the clinical treatment, it often appears insensitive to radiotherapy or resistance to chemotherapy, which will lead to poor clinical treatment effects and poor prognosis (Van Meir et al. 2010). At present, the specific pathogenesis of glioma is not very clear. Therefore, glioma has always been one of the most difficult problems in neurosurgery. Studies have shown that in addition to the effects of high-dose ionizing radiation, genetic susceptibility genes may play a certain role in the pathogenesis of glioma (Tanyıldız et al. 2016). At present, some studies on the association between genetic polymorphisms and glioma have been reported worldwide (Chen et al. 2015;

✉ Tianbo Jin
jintianbo@gmail.com

1 Clinical Laboratory, Xi'an 630 Hospital, Yanliang, Xi'an 710000, Shaanxi, China

2 Engineering Research Centre for Miniaturized Detection Systems, College of Life Science, Northwest University, Xi'an, People's Republic of China

3 The Hospital of Xi'an Shiyou University, Xi'an, People's Republic of China

4 Shaanxi Provincial Tumor Hospital, Xi'an, People's Republic of China

5 Xi'an No. 3 Hospital, The Affiliated Hospital of Northwest University, Xi'an, People's Republic of China

6 Key Laboratory of Molecular Mechanism and Intervention Research for Plateau Diseases of Tibet Autonomous Region, School of Medicine, Xizang Minzu University, Xianyang 712082, Shaanxi, China

7 Key Laboratory of Resource Biology and Biotechnology in Western China (Northwest University), Ministry of Education, School of Life Sciences, Northwest University, Xi'an 710069, Shaanxi, China

Custódio et al. 2011; He et al. 2016; Jiao et al. 2016; Shamran et al. 2014). Although these studies have let us to gain some new insights into the pathogenesis of glioma, we have not found an effective, specific, and unified method for prevention and treatment. Therefore, finding new and effective genetic markers is still very important, which will help us to judge the prognosis of glioma patients early and then conduct targeted interventions for treatment.

N-myc downstream regulated gene-1 (*NDRG1*) was cloned and isolated in 1997 for the first time and has been found in many cancers (Azuma et al. 2012), such as pancreatic cancer (Stein et al. 2004), prostate cancer (Kovacevic et al. 2011), and esophageal squamous cell carcinoma (Rabouille and Klumperman 2005). *NDRG1* has also been found to be involved in embryogenesis and development, cell growth and differentiation, lipid synthesis, stress response, immune function, and myeloid formation (Kovacevic and Richardson 2006). Most importantly, *NDRG1* may play an inhibitory role in the development of glioma and may be a potential prognostic indicator for glioma (Sun et al. 2009).

There is evidence that relatives of patients with glioma have a higher risk of glioma (Hemminki et al. 2009). And some studies have shown that the gene polymorphisms, one of genetic variation, are considered as a risk factor for glioma (Wrensch et al. 2005). The genome-wide association study of glioma has reported the association between gene polymorphisms and the risk of glioma, such as *CDKN2B*, *RTEL1*, *and PHLDB1* et al. (Shete et al. 2009). However, no research on the association between *NDRG1* gene polymorphisms and glioma risk or patient prognosis has been found. Therefore, we explored the association between 5 candidate SNPs on *NDRG1* (rs2272646 A/G, rs3779941 C/A, rs3808599 G/C, rs2977497 T/C, rs3802251 C/T) and glioma risk or patient prognosis in Chinese Han population through the experimental design of 'case–control'.

Materials and Methods

Study Subjects

In this study, 1061 participants (558 glioma patients and 503 healthy individuals) were recruited at the department of Neurosurgery at Tangdu Hospital (Xi'an, China) during the same period, and then, we conducted a study on the association between *NDRG1* SNPs and the risk of glioma. At the same time, we also explored the impact of *NDRG1* SNPs on the prognosis of patients with glioma. 558 cases were composed of glioma patients in the Department of Neurosurgery at Tangdu Hospital (Xi'an, China), and 503 healthy individuals were collected from the physical examination center of Tangdu Hospital (Xi'an, China) during the same period. All glioma patients meet the WHO diagnostic criteria for central nervous system tumors, while none of the healthy individuals has a history of cancer or central nervous system disease. All participants did not have any blood diseases. This study adopts the 'case–control' research method as a whole. In order to get the basic demographic and epidemiological information of all participants (age, gender, WHO grade, surgical operation, radiotherapy, chemotherapy, astrocytomas), we collected useful information through medical records, questionnaire surveys, and follow-up. Finally, after obtaining the informed consent of all participants, we collected peripheral blood samples from each of them for subsequent DNA extraction (blood collection for glioma patients must be done before radiotherapy, chemotherapy, and surgery). The study has been approved by the ethics committee of the Northwest University, and the follow-up work was carried out after obtaining the informed consent of all patients.

Selection and Genotyping of SNPs

Combining the relevant information of *NDRG1* gene polymorphisms in the dbSNP database, we selected candidate SNPs with an allele frequency $\geq 5\%$. Then, five SNPs on *NDRG1* were selected for our study (rs2272646 A/G, rs3779941 C/A, rs3808599 G/C, rs2977497 T/C, rs3802251 C/T). We extracted and purified the whole genome DNA according to the experimental procedures from the kit instructions (GoldMag Co. Ltd. Xi'an, China). Subsequently, we used NanoDrop 2000 to test the purity and concentration of DNA samples, and the test results showed that the OD260/280 of all DNA was between 1.8 and 2.0, indicating that the purity of DNA samples was good, which was conducive to subsequent studies. Afterwards, the extracted DNA was stored in a low-temperature refrigerator (− 80 °C) until needed for the next experiment. The primers required for this study were all designed by MassARRAY Assay Design software, and finally the MassARRAY (Agena, San Diego, CA, USA) system was used by us for genotyping.

In order to ensure the reliability and reproducibility of the experimental results, we randomly select 5% of DNA samples for repeatability testing. And the repetition rate of experimental results is > 99%.

Statistical Analysis

The Association Between SNPs on NDRG1 and the Risk of Glioma

The difference in demographic characteristics in this study was tested by SPSS 17.0 statistical software. The *p* value represents whether it is statistically significant ($p < 0.05$: statistically significant). After testing whether all candidate SNPs meet the Hardy–Weinberg balance (HWE), the correlation between the

candidate SNPs and the risk of glioma was studied. The study included overall analysis and subgroup analysis (age, gender, astrocytomas). Using wild-type alleles as a reference, the plink 1.07 online tool software was used to estimate multiple genetic models (codominant, dominant, recessive, and logarithmic addition). The analysis results of this part were all estimated based on the odds ratio (OR) and 95% confidence interval (CI) obtained by the logistic regression model adjusted by age and gender (OR 1: the factor has no effect on the occurrence of the disease; OR < 1: reduce the risk of disease; OR > 1: increase the risk of disease). Finally, we used multi-factor dimensionality reduction (MDR) to evaluate the interactions of candidate 'SNP–SNP' in the risk of glioma.

Prognosis Analysis of 558 Patients with Glioma

The overall prognosis analysis is based on SPSS 17.0 software for statistical analysis. Univariate survival analysis used the Kaplan–Meier method to calculate the median survival time and 1-year, 2-year, and 3-year survival rates of patients. The Log-rank test was used to compare survival risks. The Cox hazard proportional regression model was used to calculate hazard ratios (HRs) and 95% confidence intervals (CIs), and then, we evaluated the impact of *NDRG1* genotype on the overall survival and progression-free survival of glioma patients. We also used Kaplan–Meier method and Log-rank test to draw the corresponding survival curves of glioma patients.

All tests in this study were two-sided tests, and $p < 0.05$ was considered statistically significant.

Results

Sample Overview

This study adopted a 'case–control' experimental design. The average age of glioma patients was 40.52 ± 18.08 years, including 307 males (55%) and 251 females (45%); the average age of healthy individuals was 40.75 ± 13.99 years, including 280 males (56%) and 223 females (44%). Table 1 summarizes the demographic (age and gender) and clinical information (WHO grade, astrocytoma, surgical operation, radiotherapy status, and chemotherapy status) of the participants. We found that there was no statistical difference between the case group and the control group in gender ($p = 0.853$) and age ($p = 0.817$).

Table 1 Characteristics of patients with glioma and healthy individuals

Characteristics	Cases $n = 558$	Control $n = 503$	p
Age (years)			
Mean ± SD	40.52 ± 18.08	40.75 ± 13.99	0.817
> 40	289 (52%)	245 (49%)	
≤ 40	269 (48%)	258 (51%)	
Gender			
Male	307 (55%)	280 (56%)	0.853
Female	251 (45%)	223 (44%)	
WHO grade			
I–II	352 (63%)	–	
III–IV	206 (37%)		
WHO classification			
Astrocytoma	348		
Ependymoma	37		
Glioblastoma	38		
Oligodendrocytes astrocytoma	80		
Oligodendroglioma	19		
Others	36		
Surgical operation			
STR & NTR	175 (31%)	–	
GTR	383 (69%)		
Radiotherapy			
Conformal radiotherapy	145 (26%)	–	
Gamma knife	356 (64%)		
No	57 (10%)		
Chemotherapy			
Yes	227 (41%)	–	
No	331 (59%)		

WHO World Health Organization, *GTR* gross-total resection, *NTR* near-total resection, *STR* sub-total resection

$p < 0.05$: indicates statistical significance

Genotyping and Candidate SNPs-Related Information

5 candidate SNPs (rs2272646 A/G, rs3779941 C/A, rs3808599 G/C, rs2977497 T/C, rs3802251 C/T) on *NDRG1* were successfully genotyped. Detailed information about these five candidate SNPs is summarized in Table 2. All candidate SNPs were in line with HWE ($p > 5\%$). The results of HaploReg showed that the SNPs in this study may be regulated by many factors, including Enhancer histone marks, DNAse, Motifs changed, GRASP QTL Hits, Selected eQTL Hits, and Promoter histone marks.

Evaluation of the Correlation Between NDRG1 SNPs and Glioma Risk

Overall Analysis

The association between SNPs on *NDRG1* and glioma risk under multiple genetic models was tested based on logistic regression, and the results were adjusted by age and gender (Table 3). The results showed that among the

Table 2 The basic information and HWE about the selected SNPs of *NDRG1*

SNP ID	Call rate	Chr: position	Alleles (A/B)	MAF		HWE (*p* value)	Haploreg 4.1
				Cases	Controls		
rs2272646	99.4%	8: 134254051	A/G	0.319	0.301	0.523	Enhancer histone marks; DNAse; Motifs changed; GRASP QTL Hits; Selected eQTL Hits
rs3779941	100%	8: 134257728	C/A	0.117	0.108	0.163	Enhancer histone marks; DNAse; Motifs changed
rs3808599	100%	8: 134267886	G/C	0.156	0.180	0.172	Enhancer histone marks; DNAse; Motifs changed; Selected eQTL Hits
rs2977497	99.7%	8: 134277855	T/C	0.414	0.444	0.651	Enhancer histone marks; DNAse; Motifs changed
rs3802251	100%	8: 134305599	C/T	0.392	0.448	0.787	Promoter histone marks; Enhancer histone marks; DNAse; Motifs changed

SNP single nucleotide polymorphism, *MAF* minor allele frequency, *HWE* Hardy–Weinberg equilibrium

five candidate SNPs, rs3808599 or rs3802251 and the risk of glioma may have a certain association. Specifically, rs3808599 on *NDRG1* can reduce the risk of gliomas in homozygous (GG vs. CC, OR 0.41, CI 0.19–0.89, $p = 0.024$) and recessive models (GG vs. GC-CC, OR 0.42, CI 0.19–0.90, $p = 0.025$); rs3802251 on *NDRG1* can also reduce gliomas risk in allelic (C vs. T, OR 0.79, CI 0.67–0.94, $p = 0.008$), homozygous (CC vs. TT, OR 0.63, CI 0.44–0.90, $p = 0.011$), dominant (CC-CT vs. TT, OR 0.73, CI 0.56–0.95, $p = 0.017$), and log-additive models (OR 0.79, CI 0.66–0.94, $p = 0.008$). We did not find any evidence of the association between the remaining three candidate SNPs and glioma risk.

Age and Gender

The results showed (Table 4) that rs3808599 on *NDRG1* reduced the risk of glioma among the participants ≤ 40 years old under the homozygous model (GG vs. CC, OR 0.30, CI 0.11–0.83, $p = 0.020$) and the recessive model (GG vs. GC-CC, OR 0.29, CI 0.11–0.82, $p = 0.019$); the rs3802251 on *NDRG1* can also reduce the risk of glioma among males of the participants under heterozygous (CT vs. TT, OR 0.69, CI 0.47–1.00, $p = 0.049$) and dominant models (CC-CT vs. TT, OR 0.68, CI 0.48–0.97, $p = 0.0033$). We also found that rs3802251 only showed the ability to reduce the risk of glioma in participants > 40 years old under the allelic model (C Vs. T, OR 0.78, CI 0.61–0.99, $p = 0.049$), but the *p* value was infinitely close to the critical value (0.05). If it is inferred from the above results that rs3802251 has a significant association with the risk reduction of glioma among participants > 40 years old, the reason may be insufficient. Therefore, it is very necessary to carry out necessary verification experiments in the future. In addition, we did not find evidence that there is an association between the five candidate SNPs and the risk of glioma in the female participants.

Astrocytoma

The results showed (Table 5) that rs3802251 on *NDRG1* has a certain association with astrocytoma patients in allelic (C vs. T, OR 0.81, CI 0.67–0.97, $p = 0.023$), homozygous (CC vs. TT, OR 0.67, CI 0.46–0.99, $p = 0.043$), dominant (CC-CT vs. TT, OR 0.75, CI 0.57–0.99, $p = 0.042$), and log-additive models (OR 0.81, CI 0.68–0.98, $p = 0.031$), and it showed a risk in reduction effect (OR < 1).

WHO Grade

The results showed (supplemental Table 1) that there may be no association between the five candidate *NDRG1* SNPs and the WHO grade of glioma in Chinese Han population.

MDR Analysis

MDR analysis was used to evaluate the interactions between 'SNP–SNP'. Figure 1 can describe the interaction between 5 candidate SNPs. The blue line indicated that the candidate SNPs may have a redundant role in regulating the risk of glioma. All experimental results have been shown in Table 6: The best single-point model for predicting the risk of glioma is rs3802251 (testing accuracy = 0.539, CVC = 10/10, $p = 0.0094$); the two-site model is rs3779941, rs3802251 (testing accuracy = 0.511, CVC = 4/10, $p = 0.0003$); the three-site model is rs3779941, rs3808599, rs3802251 (testing accuracy = 0.504, CVC = 4/10, $p < 0.0001$); the four-site model is rs2272646, rs3808599, rs2977497, rs3802251 (testing accuracy = 0.511, CVC = 5/10, $p < 0.0001$); and the five-site model is rs2272646, rs3779941, rs3808599, rs2977497, rs3802251 (testing accuracy = 0.540,

Table 3 Analysis of the association between glioma and SNPs of *NDRG1*

SNP ID	Model	Genotype	Case	Control	Adjusted by age and gender	
					OR (95% CI)	*p*
rs2272646	Allele	A/G	356	299	1.09 (0.90–1.31)	0.367
	Homozygote	AA/GG	66	48	1.26 (0.84–1.90)	0.268
	Heterozygote	AG/GG	224	203	1.01 (0.78–1.31)	0.929
	Dominant	AA-AG/GG	290	251	1.06 (0.83–1.35)	0.641
	Recessive	AA/AG-GG	66	48	1.25 (0.85–1.86)	0.259
	Additive	–	–	–	1.08 (0.90–1.30)	0.385
rs3779941	Allele	C/A	131	109	1.09 (0.84–1.43)	0.512
	Homozygote	CC/AA	5	9	0.51 (0.17–1.54)	0.234
	Heterozygote	CA/AA	121	91	1.24 (0.92–1.68)	0.165
	Dominant	CC-CA/AA	126	100	1.18 (0.87–1.58)	0.286
	Recessive	CC/CA-AA	5	9	0.49 (0.16–1.48)	0.205
	Additive	–	–	–	1.09 (0.83–1.43)	0.518
rs3808599	Allele	G/C	174	181	0.84 (0.67–1.06)	0.139
	Homozygote	GG/CC	10	21	0.41 (0.19–0.89)	**0.024***
	Heterozygote	GC/CC	154	139	0.96 (0.74–1.27)	0.792
	Dominant	GG-GC/CC	164	160	0.89 (0.69–1.16)	0.393
	Recessive	GG/GC-CC	10	21	0.42 (0.19–0.90)	**0.025***
	Additive	–	–	–	0.84 (0.67–1.06)	0.141
rs2977497	Allele	T/C	460	446	0.88 (0.74–1.05)	0.156
	Homozygote	TT/CC	99	96	0.80 (0.56–1.14)	0.222
	Heterozygote	TC/CC	262	254	0.80 (0.61–1.06)	0.116
	Dominant	TT-TC/CC	361	350	0.80 (0.62–1.04)	0.096
	Recessive	TT/TC-CC	99	96	0.92 (0.67–1.25)	0.581
	Additive	–	–	–	0.88 (0.74–1.05)	0.155
rs3802251	Allele	C/T	437	451	0.79 (0.67–0.94)	**0.008***
	Homozygote	CC/TT	85	99	0.63 (0.44–0.90)	**0.011***
	Heterozygote	CT/TT	267	253	0.77 (0.59–1.01)	0.062
	Dominant	CC-CT/TT	352	352	0.73 (0.56–0.95)	**0.017***
	Recessive	CC/CT-TT	85	99	0.73 (0.53–1.01)	0.055
	Additive	–	–	–	0.79 (0.66–0.94)	**0.008***

Bold values indicate that the value is statistically significant

SNP single nucleotide polymorphisms, *OR* odds ratio, *CI* confidence interval

$p < 0.05$: indicates statistical significance

CVC = 10/10, $p < 0.0001$). Therefore, our analysis concluded that the impact of the five candidate SNPs on the risk of glioma may be interdependent.

Haplotype Analysis of NDRG1

The results of linkage disequilibrium (LD) and haplotype analysis of *NDRG1* polymorphism showed that the LD block (Fig. 2) was composed of two SNPs (rs2272646 and rs3779941). In addition, in the haplotype analysis, we also adjusted for the effects of covariates (age and gender). Haploidy frequency (case group/control group) was shown in Supplemental Table 2. The logistic regression results show that there is no haplotype significantly related to the risk of glioma.

Prognosis Analysis of 558 Patients with Glioma

Overall

A follow-up survey was conducted on 558 glioma patients in this study, and the follow-up time was 1–36 months. Based on the follow-up records, we conducted a univariate analysis between overall survival (OS) or progression-free survival (PFS) and clinical factors in 558 glioma patients. These clinical factors include: gender, age, WHO grade, surgical operation, radiotherapy status, and chemotherapy status (Table 7 and Fig. 3). Our results showed

Table 4 The SNPs of *NDRG1* associated with risk of glioma in the subgroup tests (age and gender)

SNP ID	Model	Genotype	Age, years				Gender			
			OR (95% CI)	*p*	OR (95% CI)	*p*	OR (95% CI)	*p*	OR (95% CI)	*p*
			≤40		>40		Female		Male	
rs2272646	Allele	A/G	1.17 (0.90–1.52)	0.240	1.02 (0.78–1.32)	0.897	1.00 (0.76–1.31)	0.977	1.17 (0.91–1.50)	0.216
	Homozygote	AA/GG	1.21 (0.68–2.14)	0.513	1.50 (0.81–2.78)	0.202	1.02 (0.55–1.89)	0.954	1.50 (0.86–2.60)	0.153
	Heterozygote	AG/GG	1.27 (0.88–1.85)	0.204	0.81 (0.56–1.16)	0.247	0.97 (0.66–1.43)	0.883	1.04 (0.74–1.48)	0.810
	Dominant	AA-AG/GG	1.26 (0.89–1.79)	0.197	0.91 (0.64–1.28)	0.582	0.98 (0.68–1.41)	0.916	1.13 (0.81–1.56)	0.471
	Recessive	AA/AG-GG	1.08 (0.63–1.87)	0.772	1.65 (0.91–3.00)	0.100	1.03 (0.57–1.86)	0.917	1.47 (0.86–2.50)	0.156
	Additive	–	1.15 (0.89–1.49)	0.279	1.05 (0.81–1.36)	0.737	1.00 (0.76–1.31)	0.976	1.16 (0.91–1.48)	0.234
rs3779941	Allele	C/A	0.95 (0.66–1.38)	0.803	1.29 (0.87–1.91)	0.210	1.10 (0.74–1.64)	0.642	1.09 (0.76–1.57)	0.644
	Homozygote	CC/AA	0.32 (0.08–1.22)	0.095	N/A	0.999	0.91 (0.22–3.71)	0.897	0.19 (0.02–1.62)	0.128
	Heterozygote	CA/AA	1.23 (0.79–1.92)	0.362	1.27 (0.83–1.94)	0.275	1.16 (0.73–1.83)	0.531	1.31 (0.87–1.97)	0.193
	Dominant	CC-CA/AA	1.08 (0.70–1.65)	0.732	1.30 (0.85–1.99)	0.221	1.14 (0.73–1.77)	0.573	1.21 (0.81–1.80)	0.351
	Recessive	CC/CA-AA	0.31 (0.08–1.17)	0.083	N/A	0.999	0.88 (0.22–3.59)	0.863	0.18 (0.02–1.53)	0.116
	Additive	–	0.95 (0.66–1.37)	0.784	1.33 (0.88–2.02)	0.175	1.10 (0.74–1.63)	0.649	1.09 (0.75–1.58)	0.643
rs3808599	Allele	G/C	0.78 (0.57–1.07)	0.127	0.92 (0.66–1.28)	0.622	0.88 (0.62–1.25)	0.481	0.81 (0.60–1.10)	0.180
	Homozygote	GG/CC	0.30 (0.11–0.83)	**0.020***	0.98 (0.26–3.76)	0.981	0.43 (0.13–1.47)	0.180	0.40 (0.15–1.08)	0.070
	Heterozygote	GC/CC	0.92 (0.68–1.50)	0.974	0.90 (0.61–1.32)	0.583	1.00 (0.67–1.51)	0.985	0.93 (0.65–1.34)	0.712
	Dominant	GG-GC/CC	0.87 (0.60–1.27)	0.462	0.90 (0.62–1.31)	0.594	0.94 (0.63–1.39)	0.745	0.86 (0.61–1.22)	0.395
	Recessive	GG/GC-CC	0.29 (0.11–0.82)	**0.019***	1.01 (0.27–3.85)	0.984	0.43 (0.13–1.46)	0.178	0.41 (0.15–1.09)	0.074
	Additive	–	0.79 (0.57–1.08)	0.137	0.92 (0.65–1.29)	0.629	0.88 (0.62–1.25)	0.480	0.82 (0.60–1.10)	0.182
rs2977497	Allele	T/C	0.85 (0.66–1.08)	0.178	0.93 (0.73–1.18)	0.541	0.93 (0.72–1.21)	0.588	0.85 (0.67–1.07)	0.160
	Homozygote	TT/CC	0.75 (0.45–1.25)	0.271	0.92 (0.56–1.53)	0.752	0.93 (0.54–1.59)	0.783	0.72 (0.45–1.16)	0.175
	Heterozygote	TC/CC	0.69 (0.46–1.03)	0.072	0.90 (0.61–1.32)	0.591	0.79 (0.53–1.18)	0.246	0.81 (0.56–1.18)	0.277
	Dominant	TT-TC/CC	0.71 (0.49–1.04)	0.075	0.91 (0.63–1.30)	0.593	0.82 (0.56–1.20)	0.313	0.79 (0.55–1.12)	0.180
	Recessive	TT/TC-CC	0.94 (0.61–1.47)	0.793	0.98 (0.62–1.54)	0.932	1.07 (0.66–1.73)	0.790	0.82 (0.54–1.24)	0.343
	Additive	–	0.85 (0.66–1.08)	0.185	0.95 (0.74–1.22)	0.683	0.93 (0.72–1.21)	0.586	0.85 (0.67–1.07)	0.156
rs3802251	Allele	C/T	0.81 (0.64–1.04)	0.098	0.78 (0.61–0.99)	**0.044***	0.78 (0.60–1.01)	0.059	0.80 (0.64–1.01)	0.064
	Homozygote	CC/TT	0.62 (0.38–1.03)	0.067	0.66 (0.39–1.11)	0.118	0.58 (0.34–0.99)	0.055	0.66 (0.41–1.07)	0.091
	Heterozygote	CT/TT	0.82 (0.55–1.22)	0.320	0.72 (0.49–1.05)	0.087	0.88 (0.59–1.32)	0.548	0.69 (0.47–1.00)	**0.049***
	Dominant	CC-CT/TT	0.75 (0.52–1.10)	0.144	0.70 (0.49–1.01)	0.056	0.79 (0.54–1.15)	0.222	0.68 (0.48–0.97)	**0.033***
	Recessive	CC/CT-TT	0.71 (0.45–1.10)	0.122	0.80 (0.50–1.29)	0.356	0.62 (0.38–1.01)	0.053	0.83 (0.55–1.27)	0.397
	Additive	–	0.79 (0.62–1.02)	0.067	0.79 (0.61–1.02)	0.069	0.78 (0.60–1.01)	0.062	0.79 (0.63–1.01)	0.057

Bold values indicate that the value is statistically significant

SNP single nucleotide polymorphisms, *OR* odds ratio, *CI* confidence interval

$p < 0.05$: indicates statistical significance

Table 5 The SNPs of *NDRG1* associated with risk of glioma in the subgroup tests (astrocytoma)

SNP ID	Model	Genotype	Astrocytoma (astrocytoma in case group Vs. all controls)	
			OR (95% CI)	*p*
rs2272646	Allele	A/G	1.03 (0.85–1.26)	0.764
	Homozygote	AA/GG	1.19 (0.77–1.85)	0.435
	Heterozygote	AG/GG	0.94 (0.71–1.23)	0.642
	Dominant	AA-AG/GG	0.98 (0.76–1.28)	0.909
	Recessive	AA/AG-GG	1.23 (0.80–1.87)	0.343
	Additive	–	1.04 (0.85–1.26)	0.728
rs3779941	Allele	C/A	1.09 (0.82–1.45)	0.564
	Homozygote	CC/AA	0.70 (0.23–2.13)	0.535
	Heterozygote	CA/AA	1.20 (0.87–1.66)	0.276
	Dominant	CC-CA/AA	1.16 (0.84–1.59)	0.371
	Recessive	CC/CA-AA	0.68 (0.23–2.05)	0.493
	Additive	–	1.10 (0.82–1.46)	0.535
rs3808599	Allele	G/C	0.85 (0.67–1.09)	0.203
	Homozygote	GG/CC	0.50 (0.22–1.11)	0.086
	Heterozygote	GC/CC	0.96 (0.72–1.28)	0.778
	Dominant	GG-GC/CC	0.90 (0.68–1.19)	0.458
	Recessive	GG/GC-CC	0.50 (0.23–1.11)	0.090
	Additive	–	0.86 (0.68–1.10)	0.229
rs2977497	Allele	T/C	0.85 (0.70–1.02)	0.079
	Homozygote	TT/CC	0.75 (0.51–1.09)	0.130
	Heterozygote	TC/CC	0.80 (0.60–1.07)	0.127
	Dominant	TT-TC/CC	0.78 (0.59–1.03)	0.082
	Recessive	TT/TC-CC	0.85 (0.61–1.20)	0.361
	Additive	–	0.85 (0.71–1.03)	0.094
rs3802251	Allele	C/T	0.81 (0.67–0.97)	**0.023***
	Homozygote	CC/TT	0.67 (0.46–0.99)	**0.043***
	Heterozygote	CT/TT	0.78 (0.58–1.05)	0.096
	Dominant	CC-CT/TT	0.75 (0.57–0.99)	**0.042***
	Recessive	CC/ CT-TT	0.78 (0.56–1.10)	0.156
	Additive	–	0.81 (0.68–0.98)	**0.031***

Bold values indicate that the value is statistically significant

SNP single nucleotide polymorphisms, *OR* odds ratio, *CI* confidence interval

$p < 0.05$: indicates statistical significance

that from the perspective of surgical resection methods, glioma patients with total tumor resection (OS: log-rank $p < 0.001$, HR = 0.62; PFS: log-rank $p < 0.001$, HR = 0.60) had a better prognosis than patients with non-total resection, and the result was statistically significant ($p < 0.001$). For the radiotherapy, glioma patients who have undergone gamma knife radiotherapy were associated with an increased risk of PFS (PFS: log-rank $p = 0.039$, HR = 1.40, $p = 0.041$). For the chemotherapy, compared with glioma patients who have not undergone chemotherapy, patients after chemotherapy have a better prognosis (OS: log-rank $p < 0.001$, HR = 0.70, $p < 0.001$). However, we did not find any evidence that other clinical factors (gender, age, WHO grade) were related to the prognosis of glioma patients.

Astrocytoma Patients

The results showed that female astrocytoma patients have a potential association with progression-free survival (PFS: log-rank $p = 0.029$, HR = 1.23, $p = 0.050$). As shown in Table 8 and Fig. 4a and b, patients with total tumor resection had a better prognosis than patients with non-total resection (OS: log-rank $p < 0.001$, HR = 0.62, $p < 0.001$; PFS: log-rank $p < 0.001$, HR = 0.58, $p < 0.001$). For radiotherapy (Table 8 and Fig. 4c), compared with astrocytoma patients who are not undergoing radiotherapy, the results showed that no matter what kind of radiotherapy was given, it was associated with an increased risk of PFS in astrocytoma patients (log-rank $p = 0.031$, Conformal radiotherapy: HR = 1.59, $p = 0.023$; Gamma knife: HR = 1.50, $p = 0.029$). For chemotherapy, astrocytoma patients who have undergone chemotherapy have a better prognosis (OS: log-rank $p < 0.001$, HR = 0.62, $p < 0.001$). Table 8 summarizes the experimental results after univariate analysis.

SNPs and the Prognosis of Glioma Patients (Univariate Analysis)

We evaluated the impact of five candidate SNPs on the survival rate of glioma patients. The results are shown in Table 9 and Fig. 5, and we found that rs3779941 has a potential impact on the OS and PFS of glioma patients (OS: log-rank $p = 0.006$; PFS: log-rank $p = 0.040$). At the same time, we also found an evidence that the genotype CC of rs3779941 was associated with the increased risk of OS in glioma patients (OS: HR = 3.07, 95% CI 1.27–7.44, $p = 0.013$).

SNPs and the Prognosis of Glioma Patients (Multivariate Analysis)

After Cox multivariate analysis (multivariate: gender, age, WHO grade, radiotherapy, surgical operation, chemotherapy), the results showed that the rs3779941 polymorphism was associated with prognosis of glioma patients (Table 10). Specifically, the genotype CC of rs3779941 was a risk factor that increases the risk of OS (OS: HR = 2.59, 95% CI 1.05–6.37, $p = 0.039$) in glioma patients, but there was no association with PFS (HR = 1.77, 95% CI 0.72–4.35, $p = 0.212$). There did not seem to be any association between the remaining candidate SNPs and the OS or PFS of glioma patients.

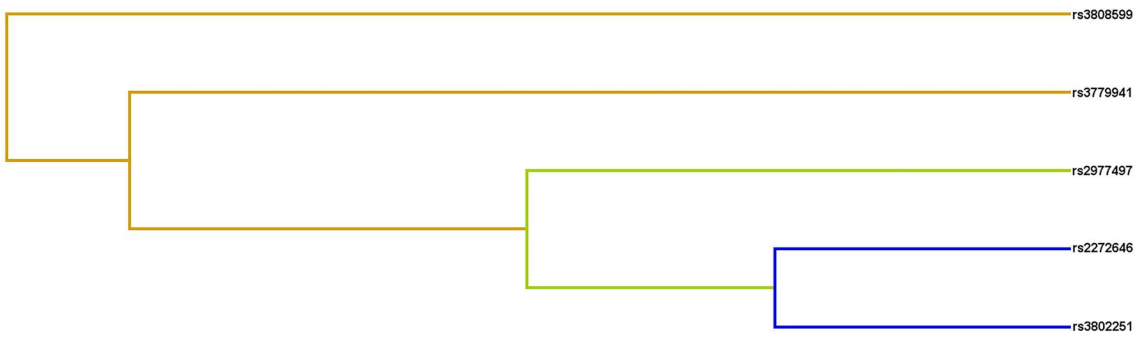

Fig. 1 Dendrogram analysis of SNP–SNP interaction (*NDRG1*). The colors in the tree diagram represent synergy (yellow) or redundancy (blue)

Table 6 SNP–SNP interaction models analyzed by the MDR method

Model	Training Bal. Acc	Testing Bal. Acc	OR (95% CI)	*p* value	CVC
rs3802251	0.539	0.539	1.42 (1.09–1.84)	**0.0094***	10/10
rs3779941, rs3802251	0.559	0.511	1.59 (1.23–2.04)	**0.0003***	4/10
rs3779941, rs3808599, rs3802251	0.575	0.504	1.82 (1.41–2.35)	**< 0.0001***	4/10
rs2272646, rs3808599, rs2977497, rs3802251	0.593	0.511	2.05 (1.59–2.63)	**< 0.0001***	5/10
rs2272646, rs3779941, rs3808599, rs2977497, rs3802251	0.610	0.540	2.38 (1.85–3.07)	**< 0.0001***	10/10

Bold values indicate that the value is statistically significant

MDR multi-factor dimensionality reduction, *Bal. Acc.* balanced accuracy, *CVC* cross-validation consistency, *OR* odds ratio, *95% CI* 95% confidence interval

p values were calculated using χ^2 tests

$p < 0.05$: indicates statistical significance

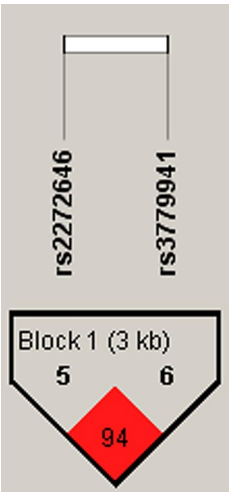

Fig. 2 Haplotype block map for 2 SNPs in *NDRG1* gene. The numbers inside the diamonds indicate the *D′* for pairwise analyses

SNPs and the Prognosis of Astrocytoma Patients (Multivariate Analysis)

Finally, we also performed the association analysis between *NDRG1* gene polymorphisms and the prognosis of astrocytoma patients. The results showed (Table 10) that the genotype CC of rs3779941 was a risk factor that increased the risk of OS in astrocytoma patients (OS: HR = 2.63, 95% CI 1.06–6.56, $p = 0.038$), but there was no association with PFS (HR = 1.78, 95% CI 0.72–4.42, $p = 0.211$). There did not seem to be any association between the remaining candidate SNPs and the OS or PFS of astrocytoma patients.

Discussion

Glioma is the tumor with the highest incidence and the worst prognosis among primary brain tumors, posing a great threat to human health. With the development of sequencing technology and genome-wide association studies (GWAS), more and more studies have proved that in addition to external factors such as high-dose ionizing radiation, genetic susceptibility genes also play a certain role in the occurrence and development of glioma (Ostrom et al. 2014; Tanyıldız et al. 2016), such as *POLR3B*, *VTI1A*, *ZBTB16*, *ETFA*, etc. (Kinnersley et al. 2018). Up to now, there is no report about the association between *NDRG1* gene polymorphisms and the occurrence and prognosis of glioma. However, studies have shown that *NDRG1* is necessary to inhibit the occurrence of glioma (Ma et al. 2015). This study was the first to explore the relationship

Table 7 Univariate analysis of the influence of clinical factors on glioma patient OS and PFS

Factors	Total	Event	OS				PFS			
			Log-rank *p*	SR (1/3 year)	HR (95% CI)	*p*	Log-rank *p*	SR (1 /3 year)	HR (95% CI)	*p*
Gender										
Male	307	273	0.314	0.334/0.079	1.00		0.214	0.199/0.091	1.00	
Female	251	226		0.315/0.090	1.09 (0.91–1.3)	0.356		0.149/0.093	1.11 (0.93–1.32)	0.266
Age										
< 40 years	248	214	0.059	0.361/0.114	1.00		0.100	0.198/0.111	1.00	
≥ 40 years	310	285		0.297/0.061	1.17 (0.98–1.40)	0.084		0.159/0.069	1.14 (0.96–1.37)	0.140
WHO grade										
I–II	352	310	0.273	0.328/0.101	1.00		0.271	0.184/0.099	1.00	
III–IV	206	189		0.320/0.066	1.10 (0.92–1.32)	0.315		0.164/0.069	1.10 (0.91–1.31)	0.324
Surgical operation										
STR & NTR	175	173	**< 0.001***	0.206/–	1.00		**< 0.001***	0.012/–	1.00	
GTR	383	326		**0.380/0.118**	**0.62 (0.52–0.76)**	**< 0.001***		**0.252/0.121**	**0.60 (0.49–0.72)**	**< 0.001***
Radiotherapy										
No	57	46	0.346	0.456/–	1.00		0.061	0.204/–	1.00	
Conformal radiotherapy	145	119		0.252/0.147	1.12 (0.80–1.58)	0.509		0.217/0.156	1.42 (1.00–2.01)	0.052
Gamma knife	356	334		0.334/0.052	0.21 (0.89–1.65)	0.220		**0.158/0.048**	**1.40 (1.01–1.92)**	**0.041***
Chemotherapy										
No	331	309	**< 0.001***	0.278/0.029	1.00		0.054	0.170/0.060	1.00	
Yes	227	190		**0.395/0.143**	**0.70 (0.58–0.84)**	**< 0.001***		0.188/0.140	0.85 (0.71–1.02)	0.085

Bold values indicate that the value is statistically significant

OS overall survival, *PFS* progression-free survival, *SR* survival rate, *HR* hazard ratio, *95% CI* 95% confidence interval

$p < 0.05$: indicates statistical significance

Log-rank *p* values were calculated using the Chi-Square test

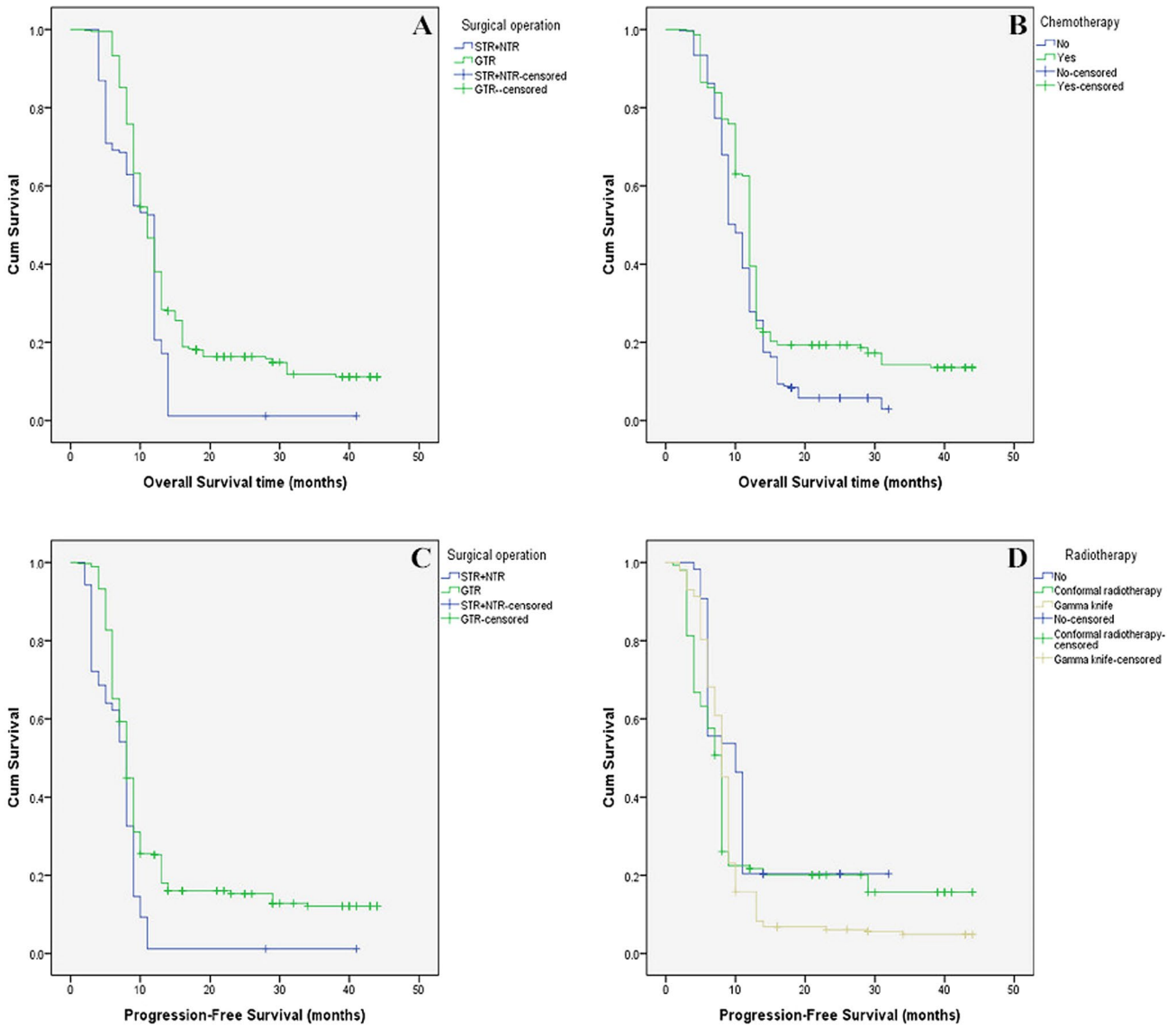

Fig. 3 Kaplan–Meier curves for overall survival and progression-free survival according to the glioma patients with different clinical factors (**a** OS according to surgical operation; **b** OS according to chemotherapy status; **c** PFS according to surgical operation; **d** PFS according to radiotherapy status)

between the five polymorphisms of *NDRG1* (rs2272646 A/G, rs3779941 C/A, rs3808599 G/C, rs2977497 T/C, rs3802251 C/T) and the genetic risk of glioma or the prognosis of patients in the Chinese Han population. And as far as we know, this study is the first to find that the *NDRG1* SNPs (rs3779941, rs3808599, and rs3802251) are potentially associated with glioma susceptibility or prognosis. In addition, in the prognosis analysis of glioma patients, we found that surgical operation, radiotherapy, and chemotherapy are the key factors for the prognosis of glioma patients in the Chinese Han population.

It has been found that *NDRG1* plays an important role in regulating the pathogenesis/molecular mechanisms of tumor cells. Based on previous studies (Byun et al. 2018; Ito et al. 2020), *NDRG1* is found to be responsible for the regulation on cellular proliferation, apoptosis, invasion, migration, and metastasis in tumor tissues. At present, *NDRG1* has been proposed as a tumor suppressor gene in a variety of cancers, including breast cancer (Bandyopadhyay et al. 2004), colon cancer (Guan et al. 2000), and prostate cancer (Bandyopadhyay et al. 2003). And *NDRG1* is also necessary for inhibiting the occurrence of glioma (Ito et al. 2020; Ma et al. 2015). Sun et al. found that the expression level of *NDRG1* in high-grade glioma tissue is relatively lower than that in normal brain tissue or low-grade glioma tissue (Sun et al. 2009). These research results prompted that *NDRG1* may

Table 8 Univariate analysis of the influence of clinical factors on astrocytoma patient OS and PFS

Factors	Total	Event	OS				PFS			
			Log-rank *p*	SR (1 /3 year)	HR (95% CI)	*p*	Log-rank *p*	SR (1 /3 year)	HR (95% CI)	*p*
Gender										
Male	237	207	0.073	0.350/0.091	1.00		**0.029***	0.222/0.102	1.00	
Female	191	173		0.304/0.071	1.18 (0.97–1.45)	0.101		**0.131/0.076**	**1.23 (1.00–1.50)**	**0.050***
Age										
< 40 years	185	158	0.177	0.341/0.110	1.00		0.273	0.196/0.119	1.00	
≥ 40 years	243	222		0.321/0.062	1.14 (0.93–1.40)	0.217		0.171/0.069	1.11 (0.90–1.36)	0.325
WHO grade										
I–II	297	260	0.244	0.340/0.104	1.00		0.264	0.189/0.113	1.00	
III–IV	131	120		0.305/0.049	1.13 (0.91–1.40)	0.286		0.165/0.057	1.12 (0.90–1.39)	0.317
Surgical operation										
STR & NTR	133	132	**< 0.001***	0.211/–	1.00		**< 0.001***	0.008/–	1.00	
GTR	295	248		**0.383/0.117**	**0.62 (0.50–0.77)**	**< 0.001***		**0.261/0.129**	**0.58 (0.47–0.72)**	**< 0.001***
Radiotherapy										
No	44	35	0.337	0.500/–	1.00		**0.031***	0.524/–	1.00	
Conformal radiotherapy	112	93		0.250/0.128	1.23 (0.83–1.82)	0.303		**0.218/0.137**	**1.59 (1.07–2.37)**	**0.023***
Gamma knife	272	252		0.335/0.058	1.27 (0.89–1.82)	0.180		**0.162/0.063**	**1.50 (1.04–2.16)**	**0.029***
Chemotherapy										
No	257	239	**< 0.001***	0.280/0.000	1.00		0.090	0.172/0.059	1.00	
Yes	171	141		**0.404/0.143**	**0.68 (0.55–0.84)**	**< 0.001***		0.197/0.147	0.85 (0.69–1.05)	0.128

Bold values indicate that the value is statistically significant

OS overall survival, *PFS* progression-free survival, *SR* survival rate, *HR* hazard ratio, *95% CI* 95% confidence interval

$p < 0.05$: indicates statistical significance;

Log-rank *p* values were calculated using the Chi-Square test

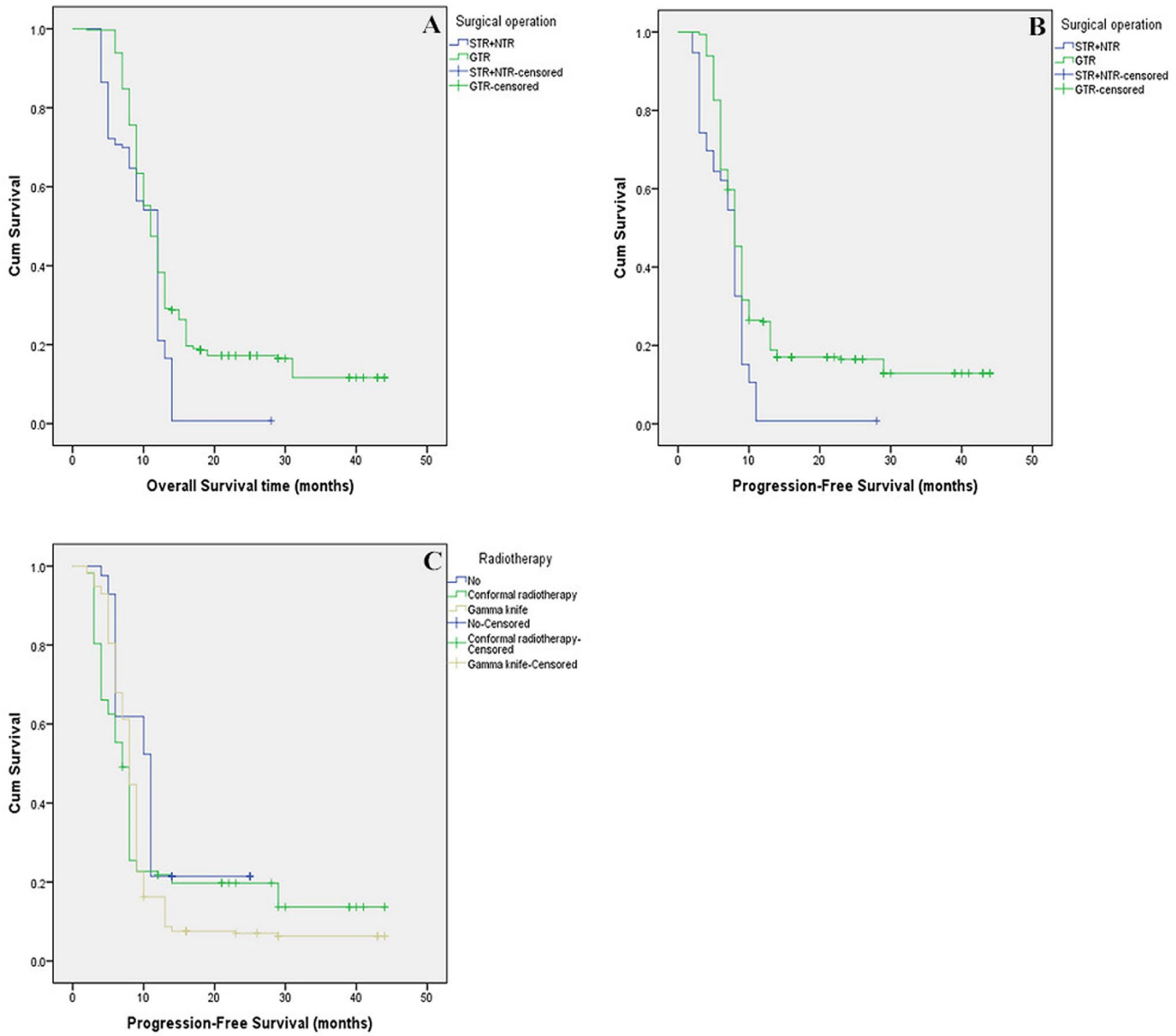

Fig. 4 Kaplan–Meier curves for overall survival and progression-free survival according to the astrocytoma patients with different clinical factors (**a** OS according to surgical operation; **b** PFS according to surgical operation; **c** PFS according to radiotherapy status)

be an internal regulator that can affect the occurrence and development of glioma, and the expression of *NDRG1* may play a very important role in the progression and prognosis of glioma.

In this study, we found that rs3808599 on *NDRG1* can reduce the risk of glioma in homozygous and recessive models, whether in the overall participants (homozygous: OR 0.41; recessive: OR 0.42) or in the participants ≤ 40 years old (homozygous: OR 0.30; recessive: OR 0.29); rs3802251 on *NDRG1* can significantly reduce the risk of glioma in the overall participants, male participants, and astrocytoma patients under variety of genetic models; the prognostic analysis after the follow-up investigation found that rs3779941 on *NDRG1* was significantly associated with the prognosis of glioma patients in our study. At the same time, we also found that the three candidate SNPs (rs3779941, rs3808599 and rs3802251), which are potentially associated with the risk or prognosis of glioma in this study, are all located in the intron region. And there have been several studies suggesting that mutants located in the intron region can disrupt transcriptional regulatory motifs by affecting gene expression, which will affect the occurrence and development of diseases (Chang et al. 2019; Vaz-Drago et al. 2017; Zhao et al. 2012). Combined with the results of this study, we speculate that rs3779941, rs3808599, and rs3802251 may affect the occurrence and development of glioma by affecting the gene expression of *NDRG1* and disrupting the transcriptional regulatory motifs.

Table 9 Univariate analysis of the association between SNPs in *NDRG1* and glioma patient OS and PFS

SNPs	Genotype	OS				PFS			
		Log-rank *p*	SR (1 /3 year)	HR (95% CI)	*p*	Log-rank *p*	SR (1 /3 year)	HR (95% CI)	*p*
rs2272646	GG	0.240	0.321/0.103	1.00		0.445	0.178/0.100	1.00	
	AG		0.311/0.051	1.09 (0.90–1.31)	0.390		0.146/0.057	1.04 (0.86–1.26)	0.676
	AA		0.394/0.120	0.87 (0.65–1.16)	0.334		0.277/–	0.88 (0.66–1.17)	0.376
rs3779941	AA	**0.006***	0.344/0.089	1.00		**0.040***	0.190/0.096	1.00	
	CA		0.273/–	1.20 (0.97–1.49)	0.090		0.134/–	1.22 (0.99–1.51)	0.068
	CC		0.000/–	**3.07 (1.27–7.44)**	**0.013***		–/–	1.91 (0.79–4.61)	0.153
rs3808599	CC	0.102	0.349/0.096	1.00		0.105	0.193/0.102	1.00	
	GC		0.273/0.062	1.19 (0.98–1.11)	0.089		0.138/0.051	1.19 (0.97–1.44)	0.089
	GG		0.200/–	1.43 (0.76–2.69)	0.264		0.100/–	1.38 (0.74–2.59)	0.316
rs2977497	CC	0.202	0.359/0.094	1.00		0.096	0.192/0.105	1.00	
	TC		0.311/0.096	1.05 (0.86–1.27)	0.661		0.183/0.090	1.03 (0.84–1.25)	0.783
	TT		0.303/0.032	1.23 (0.96–1.58)	0.108		0.131/–	1.27 (0.98–1.63)	0.066
rs3802251	TT	0.895	0.325/0.071	1.00		0.910	0.171/0.076	1.00	
	CT		0.326/0.100	0.96 (0.79–1.16)	0.679		0.187/0.096	0.97 (0.80–1.18)	0.787
	CC		0.324/0.079	0.99 (0.76–1.30)	0.960		0.158/–	1.02 (0.78–1.33)	0.882

Bold values indicate that the value is statistically significant

OS overall survival, *PFS* progression-free survival, *SR* survival rate, *HR* hazard ratio, *95% CI* 95% confidence interval

$p < 0.05$: indicates statistical significance;

Log-rank *p* values were calculated using the Chi-Square test

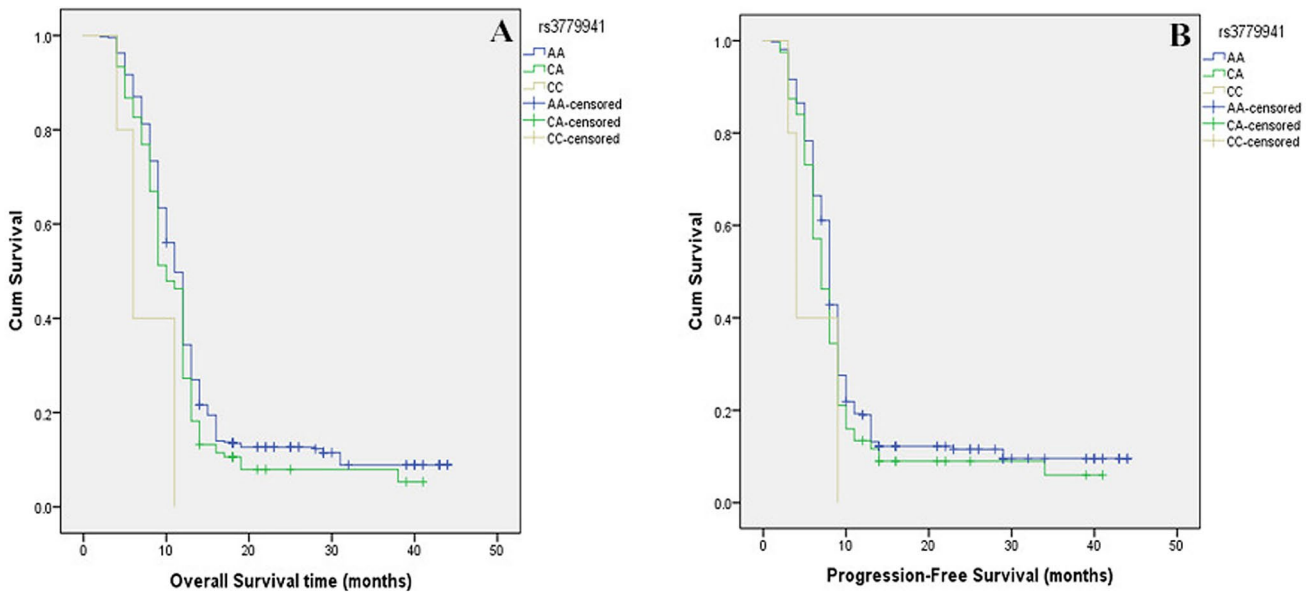

Fig. 5 Glioma patient survival based on *NDRG1* rs3779941 polymorphism. Kaplan–Meier survival curves are plotted for overall and progression-free survival (**a** OS based on *NDRG1* rs3779941 polymorphism; **b** PFS based on *NDRG1* rs3779941 polymorphism)

More importantly, studies have shown that *NDRG1* can inhibit the proliferation and invasion of glioma cells, and overexpressed *NDRG1* will inhibit the growth of glioma tumors in vivo (Ma et al. 2015). It follows that *NDRG1* inhibits the proliferation and invasion of glioma cells and other cellular behaviors, which rs3779941, rs3808599, and rs3802251 of NDRG1 may play a certain role. Our study has provided new ideas for the diagnosis and prognosis analysis of clinical glioma. Perhaps from the viewpoint of 'how rs3779941, rs3808599 and rs3802251 affect the expression of *NDRG1* in glioma cells', we will have the

Table 10 Multivariate analysis of the association between SNPs of *NDRG1* and glioma patient OS and PFS (overall and astrocytoma)

SNPs	Genotype	OS		PFS	
		HR (95% CI)	*p*	HR (95% CI)	*p*
Overall analysis					
rs2272646	GG	1.00		1.00	
	AG	0.97 (0.8–1.17)	0.742	0.95 (0.78–1.15)	0.577
	AA	0.86 (0.64–1.16)	0.330	0.89 (0.66–1.20)	0.447
rs3779941	AA	1.00		1.00	
	CA	1.13 (0.91–1.40)	0.260	1.13 (0.92–1.41)	0.248
	CC	**2.59 (1.05–6.37)**	**0.039***	1.77 (0.72–4.35)	0.212
rs3808599	CC	1.00		1.00	
	GC	1.13 (0.93–1.38)	0.211	1.13 (0.93–1.38)	0.232
	GG	1.22 (0.64–2.330	0.538	1.16 (0.61–2.20)	0.656
rs2977497	CC	1.00		1.00	
	TC	1.05 (0.86–1.28)	0.625	1.01 (0.83–1.23)	0.911
	TT	1.19 (0.92–1.54)	0.184	1.20 (0.93–1.56)	0.156
rs3802251	TT	1.00		1.00	
	CT	1.04 (0.85–1.26)	0.705	1.06 (0.87–1.29)	0.565
	CC	1.04 (0.79–1.37)	0.775	1.06 (0.81–1.39)	0.688
Astrocytoma patients					
rs2272646	GG	1.00		1.00	
	AG	0.92 (0.74–1.14)	0.453	0.93 (0.74–1.15)	0.490
	AA	0.83 (0.59–1.17)	0.293	0.89 (0.63–1.25)	0.490
rs3779941	AA	1.00		1.00	
	CA	0.64 (1.06–0.83)	0.642	1.04 (0.81–1.34)	0.746
	CC	**2.63 (1.06–6.56)**	**0.038***	1.78 (0.72–4.42)	0.211
rs3808599	CC	1.00		1.00	
	GC	1.04 (0.83–1.31)	0.748	1.03 (0.82–1.29)	0.830
	GG	1.25 (0.63–2.50)	0.525	1.15 (0.58–2.28)	0.700
rs2977497	CC	1.00		1.00	
	TC	1.07 (0.85–1.35)	0.551	1.04 (0.83–1.31)	0.734
	TT	1.08 (0.80–1.45)	0.636	1.12 (0.83–1.51)	0.467
rs3802251	TT	1.00		1.00	
	CT	1.04 (0.83–1.31)	0.728	1.06 (0.84–1.33)	0.628
	CC	1.07 (0.79–1.45)	0.660	1.10 (0.81–1.49)	0.535

Bold values indicate that the value is statistically significant

OS overall survival, *PFS* progression-free survival, *SR* survival rate, *HR* hazard ratio, *95% CI* 95% confidence interval

$p < 0.05$: indicates statistical significance;

Log-rank *p* values were calculated using the Chi-Square test

opportunity to understand the specific molecular mechanism of *NDRG1* in risk/prognosis of glioma.

Many studies have found that WHO grade is an important factor in the survival rate of glioma patients, and it is often used by doctors to predict the survival rate of glioma patients (Desjardins et al. 2018; Liu et al. 2018; Rasmussen and Hansen 2017). However, our results showed that the five candidate *NDRG1* SNPs were not associated with the WHO grade in glioma patients. Our results may indicate that the five candidate *NDRG1* SNPs did not play any role in the effect of WHO grade on the survival rate of Chinese Han glioma patients. We speculate that the reason for the result may be due to the difference of the genetic background or the small sample size. But this is only speculation, and we need further experiments to verify it. In this study, we also found that *NDRG1* rs3802251 was associated with the risk of astrocytoma in multiple genetic models (Table 5). However, the upper limit of 95% CI is all close to 1.0, and the *p* value is still < 0.05. We speculate that the reason may be the lack of samples in the subgroup. Therefore, it is necessary to expand the sample size to verify the correlation between

NDRG1 rs3802251 and the susceptibility of astrocytoma, which will make the results of this study more reliable.

There are inevitably several shortcomings in our research. On the one hand, enlarging the sample size and selection range is necessary in the following research. On the other hand, this study is only a preliminary research. Therefore, in order to clearly clarify the molecular mechanism of how the *NDRG1* SNPs affect the risk or prognosis of glioma, it is necessary to further explore how these variants (rs3779941, rs3808599 and rs3802251) affect the expression of *NDRG1*. It will help us to understand the mechanism of *NDRG1* genetic polymorphism in the occurrence and development of glioma. Despite the abovementioned deficiencies in this study, the results of our study have provided data supplement for the risk assessment of glioma in Chinese Han population.

Conclusion

In summary, the results of this study showed that the *NDRG1* gene polymorphisms have a potential association with the risk or prognosis of glioma in the Chinese Han population, which provides new ideas for the risk assessment and prognosis evaluation of glioma in the Chinese Han population.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10571-021-01075-6.

Acknowledgements We thank all authors for their contributions and support. We are also grateful to the Department of Neurosurgery at Tangdu Hospital and all participants for providing us with blood samples.

Author Contributions TJ conceived and designed the experiments; YY and CC collected samples; YC and LY performed the experiments; GS and PC analyzed the data; LL and HF contributed reagents/materials/analysis tools; YC and YY drafted the paper; and YY revised the paper.

Funding This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Data Availability The datasets used and analyzed in the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical Approval This study was conducted under the standard approved by the ethics committee of the Northwest University and conformed to the ethical principles for medical research involving humans of the World Medical Association Declaration of Helsinki.

Consent to Participate All participants signed informed consent forms before participating in this study.

Consent to Publication All authors agreed to publish the manuscript.

References

Azuma K et al (2012) NDRG1/Cap43/Drg-1 may predict tumor angiogenesis and poor outcome in patients with lung cancer. J Thorac Oncol Off Publ Int Assoc Study Lung Cancer 7:779–789. https://doi.org/10.1097/JTO.0b013e31824c92b4

Bandyopadhyay S et al (2003) The Drg-1 gene suppresses tumor metastasis in prostate cancer. Cancer Res 63:1731–1736

Bandyopadhyay S et al (2004) Role of the putative tumor metastasis suppressor gene Drg-1 in breast cancer progression. Oncogene 23:5675–5681. https://doi.org/10.1038/sj.onc.1207734

Byun JW, An HY, Yeom SD, Lee SJ, Chung HY (2018) NDRG1 and FOXO1 regulate endothelial cell proliferation in infantile haemangioma. Exp Dermatol 27:690–693. https://doi.org/10.1111/exd.13541

Chang CY, Perng CL, Cheng SN, Hu SH, Wu TY, Lin SY, Chen YC (2019) Deep intronic variant c.5999-277G>A of F8 gene may be a hot spot mutation for mild hemophilia A patients without mutation in exonic DNA. Eur J Haematol 103:47–55. https://doi.org/10.1111/ejh.13242

Chen P, Chen C, Chen K, Xu T, Luo C (2015) Polymorphisms in IL-4/IL-13 pathway genes and glioma risk: an updated meta-analysis. Tumour Biol 36:121–127. https://doi.org/10.1007/s13277-014-2895-8

Custódio AC et al (2011) Analysis of the polymorphisms XRCC1Arg194Trp and XRCC1Arg399Gln in gliomas. Genet Mol Res GMR 10:1120–1129. https://doi.org/10.4238/vol10-2gmr1125

Desjardins A et al (2018) Recurrent glioblastoma treated with recombinant poliovirus. N Engl J Med 379:150–161. https://doi.org/10.1056/NEJMoa1716435

Guan RJ, Ford HL, Fu Y, Li Y, Shaw LM, Pardee AB (2000) Drg-1 as a differentiation-related, putative metastatic suppressor gene in human colon cancer. Cancer Res 60:749–755

Gupta A, Dwivedi T (2017) A simplified overview of World Health Organization classification update of central nervous system tumors 2016. J Neurosci Rural Pract 8:629–641. https://doi.org/10.4103/jnrp.jnrp_168_17

He X et al (2016) The relationship between polymorphisms of XRCC5 genes with astrocytoma prognosis in the Han Chinese population. Oncotarget 7:85283–85290. https://doi.org/10.18632/oncotarget.13297

Hemminki K, Tretli S, Sundquist J, Johannesen TB, Granström C (2009) Familial risks in nervous-system tumours: a histology-specific analysis from Sweden and Norway. Lancet Oncol 10:481–488. https://doi.org/10.1016/s1470-2045(09)70076-2

Ito H et al (2020) Bidirectional regulation between NDRG1 and GSK3β controls tumor growth and is targeted by differentiation inducing factor-1 in glioblastoma. Cancer Res 80:234–248. https://doi.org/10.1158/0008-5472.can-19-0438

Jiao K, Qin J, Zhao Y, Zhang H (2016) Genetic effects of XRCC4 and ligase IV genes on human glioma. NeuroReport 27:1024–1030. https://doi.org/10.1097/wnr.0000000000000649

Kinnersley B, Houlston RS, Bondy ML (2018) Genome-wide association studies in glioma. Cancer Epidemiol Biomark Prev 27:418–428. https://doi.org/10.1158/1055-9965.epi-17-1080

Kovacevic Z, Richardson DR (2006) The metastasis suppressor, Ndrg-1: a new ally in the fight against cancer. Carcinogenesis 27:2355–2366. https://doi.org/10.1093/carcin/bgl146

Kovacevic Z, Sivagurunathan S, Mangs H, Chikhani S, Zhang D, Richardson DR (2011) The metastasis suppressor, N-myc

downstream regulated gene 1 (NDRG1), upregulates p21 via p53-independent mechanisms. Carcinogenesis 32:732–740. https://doi.org/10.1093/carcin/bgr046

Liu G, Jiang R, Xu C, Zhou J, Liu F, He Z, Liu Z (2018) Survival analysis for high-grade glioma patients who received comprehensive treatment. Zhong nan da xue xue bao Yi xue ban = J Cent South Univ Med Sci 43:388–393. https://doi.org/10.11817/j.issn.1672-7347.2018.04.008

Ma W, Na M, Tang C, Wang H, Lin Z (2015) Overexpression of N-myc downstream-regulated gene 1 inhibits human glioma proliferation and invasion via phosphoinositide 3-kinase/AKT pathways. Mol Med Rep 12:1050–1058. https://doi.org/10.3892/mmr.2015.3492

McNeill KA (2016) Epidemiology of brain tumors. Neurol Clin 34:981–998. https://doi.org/10.1016/j.ncl.2016.06.014

Ostrom QT et al (2014) The epidemiology of glioma in adults: a "state of the science" review. Neuro-Oncology 16:896–913. https://doi.org/10.1093/neuonc/nou087

Rabouille C, Klumperman J (2005) Opinion: the maturing role of COPI vesicles in intra-Golgi transport. Nat Rev Mol Cell Biol 6:812–817. https://doi.org/10.1038/nrm1735

Rasmussen BK, Hansen S (2017) Epidemiology of glioma: clinical characteristics, symptoms, and predictors of glioma patients grade I–IV in the the Danish Neuro-Oncology Registry. J Neurooncol 135:571–579. https://doi.org/10.1007/s11060-017-2607-5

Shamran HA et al (2014) Impact of single nucleotide polymorphism in IL-4, IL-4R genes and systemic concentration of IL-4 on the incidence of glioma in Iraqi patients. Int J Med Sci 11:1147–1153. https://doi.org/10.7150/ijms.9412

Shete S et al (2009) Genome-wide association study identifies five susceptibility loci for glioma. Nat Genet 41:899–904. https://doi.org/10.1038/ng.407

Stein S et al (2004) NDRG1 is necessary for p53-dependent apoptosis. J Biol Chem 279:48930–48940. https://doi.org/10.1074/jbc.M400386200

Sun B, Chu D, Li W, Chu X, Li Y, Wei D, Li H (2009) Decreased expression of NDRG1 in glioma is related to tumor progression and survival of patients. J Neurooncol 94:213–219. https://doi.org/10.1007/s11060-009-9859-7

Tanyıldız HG et al (2016) Are the methylenetetrahydrofolate reductase 1298 and 677 gene polymorphisms related to optic glioma and hamartoma risk in neurofibromatosis type 1 patients? Turk J Pediatr 58:152–158. https://doi.org/10.24953/turkjped.2016.02.005

Van Meir EG, Hadjipanayis CG, Norden AD, Shu HK, Wen PY, Olson JJ (2010) Exciting new advances in neuro-oncology: the avenue to a cure for malignant glioma. CA Cancer J Clin 60:166–193. https://doi.org/10.3322/caac.20069

Vaz-Drago R, Custódio N, Carmo-Fonseca M (2017) Deep intronic mutations and human disease. Hum Genet 136:1093–1111. https://doi.org/10.1007/s00439-017-1809-4

Wrensch M, Fisher JL, Schwartzbaum JA, Bondy M, Berger M, Aldape KD (2005) The molecular epidemiology of gliomas in adults. Neurosurg Focus 19:E5. https://doi.org/10.3171/foc.2005.19.5.6

Zhao H et al (2012) An intronic variant associated with systemic lupus erythematosus changes the binding affinity of Yinyang1 to downregulate WDFY4. Genes Immun 13:536–542. https://doi.org/10.1038/gene.2012.33