Effects of the ANXA6 polymorphisms on glioma risk and patients prognosis

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Abstract

BACKGROUND Glioma is the most frequent malignant primary brain tumor, and the outcomes for patients with glioma remain poor. The purpose of this study is to explore the association between ANXA6 polymorphisms and glioma risk as well as the prognosis of glioma patients in the Chinese Han population.

METHODS We selected nine single-nucleotide polymorphisms (SNPs) in ANXA6 which were genotyped by Agena MassARRAY from 593 glioma patients and 589 healthy controls. The odds ratio (OR) and 95% confidence interval (CI) were calculated by logistic regression analysis to evaluate the association SNPs with glioma risk. The association between polymorphisms and survival of glioma patient were evaluated using the log-rank test, Kaplan-Meier and Cox regression analysis.

RESULTS: Overall analysis found that rs3762993 was significantly associated with an increased glioma risk. Stratification analysis found that rs11960458 was strongly associated with an increased risk of glioma in age >41; rs3762993 was also found to be associated an increased with glioma risk in age >41, ≤41, male and low-grade glioma; but rs4958892 was associated with a decreased risk of glioma in age>41 and male. Interestingly, rs11960458 and rs888988 were correlated with poor prognosis of glioma patient. Furthermore, age, extent of resection and chemotherapy were found to be key prognostic factors in survival of glioma patients.

CONCLUSIONS In conclusion, our results indicated that ANXA6 polymorphisms were associated with glioma susceptibility and prognosis. Further studies are required to confirm the results and elucidate the mechanisms of the ANXA6 polymorphisms affect the glioma risk and prognosis.

Background

Glioma is the most frequent central nervous system (CNS) tumor comprising approximately 80% of malignant primary brain tumors[1]. Global cancer statistics in 2018 estimated that 296,851 new cases were diagnosed and 241,037 individuals died from brain and CNS tumor [2]. In China, it has reported about 101,600 newly brain and CNS tumor cases (52,300 males and 49,300 females) and 61,000 deaths (35,800 males and 25,200 females) in 2015 [3]. Gliomas are classified by World Health Organization (WHO) into four grades based on degree of aggressiveness: grade I (pilocytic astrocytomas), grade II (diffuse infiltrating low-grade gliomas), grade III (anaplastic gliomas), and grade IV (glioblastomas multiforme) [4]. Currently, the etiology of glioma has not been completely understood. Despite comprehensive multimodal treatments (surgery, radiotherapy and chemotherapy) have been adopted, the outcomes for patients with glioma remain poor. There are increasing evidences demonstrated that several risk factors such as age and gender, extent of resection, radiotherapy, chemotherapy, and histological grade, tumor size and range have been identified as potential contributors to the prognosis of glioma[5, 6]. Therefore, the identification of new therapeutic targets and new diagnostic and prognostic biomarkers remains crucial for glioma-tailored medicine. Recent association studies have identified that several genetic polymorphisms are associated with the susceptibility to gliomas and also affect its prognosis [7–9].

The annexin A6 (AnxA6) gene is located on human chromosome 5q32-q34 coding annexin A6. Annexin A6 belongs to a member of a highly conserved protein family characterized by their calcium (Ca^{2+})-dependent binding to phospholipids. AnxA6 is predominantly found at the plasma membrane and endosomal compartments. It acts as a multifunctional scaffold protein[10], recruiting signaling proteins [11], modulating cholesterol [12] and membrane transport and influencing actin dynamics [13], participating in many biological processes, including cell proliferation [14], survival [15], differentiation [16], inflammation, membrane repair [17] and viral infection[18]. AnxA6 exhibits dual functions in cancer, acting either as a tumor suppressor or promoter, depending on the type of cancer and the degree of malignancy [19].

It has been reported that the *ANXA5* polymorphisms were associated with the risk of glioma as well as the prognosis of glioma patients [20]. However, little is known about the association between polymorphisms in the *ANXA6* gene and susceptibility to glioma as well as the prognosis of glioma patients. In this study, we conducted a case-control study consisted of 593 glioma patients and 589 healthy controls to investigate whether the nine polymorphisms in the *ANXA6* gene are associated with the risk of glioma in the Chinese population, and evaluate the prognostic values of *ANXA6* polymorphisms in patients with glioma.

Materials And Methods

Study population

This case-control study consisted of 593 glioma patients and 589 healthy controls were randomly obtained from the department of Neurosurgery at Tangdu Hospital of The Fourth Military Medical University, Xian City, China. All cases recruited in this study had newly diagnosed and histologically confirmed glioma by at least 2 senior neuropathologists according to the World Health Organization (WHO) classification in 2007. The controls were randomly selected from people who requested general health examinations in the same hospital during the same period. Controls were required to be without any history of any type of cancer, and no diseases related to brain and central nervous system, and frequency matched by sex and age. All participants were unrelated Chinese ethnic background.

Follow-up

Clinical follow-up regarding the genetic status of glioma patients was performed in single-blind fashion with the end point of cardiac death. All the patients were followed-up every month by telephone, outpatient visits, and writing communication with patients or their families. Overall survival (OS) was

measured from the date of diagnosis to death or the last follow-up. Progress-free survival (PFS) was calculated from the date of the pathologically confirmed to the progression of the disease, death without progression, or last clinical follow-up.

Data collection

The demographic and clinical information were collected and regularly updated for the patients with glioma through medical records, questionnaires and follow-up. These data included gender, age, date of diagnosis, histologic type, WHO grade, surgical methods, extent of resection, treatment with radiotherapy and/or chemotherapy, date of last follow-up, status of patients (living/deceased).

DNA extraction

The peripheral blood samples were collected from all subjects before radiotherapy and chemotherapy therapy or surgery into the ethylene diamine tetraacetic acid (EDTA)-containing vacutainers, and stored at -80°C until further DNA extraction. Genomic DNA of the samples was extracted using the GoldMag-Mini Whole Blood Genomic DNA Purification Kit (GoldMag. Co. Ltd., Xi'an, China). The purity and concentration of the extracted DNA were determined using a spectrophotometer by absorbance measurements at 260nm and 280nm (NanoDrop 2000; Thermo Fisher Scientific, Waltham, MA, USA). The extracted DNA was stored at -20°C until further SNPs genotype.

SNPs selection and genotyping

Nine SNPs (rs11960458, rs4958892, rs78243462, rs4346760, rs4958897, rs3762993, rs9324677, rs13185706, and rs888988) in the *ANXA6* gene were selected with minor allele frequency (MAF) $>5\%$ in the global population from the HapMap database. We used the Agena Bioscience Assay Design Suite V2.0 software (<https://agenacx.com/online-tools/>) to design PCR amplification and extension primers of the nine SNPs. Genotyping of the nine *ANXA6* polymorphisms were performed using the Agena MassARRAY platform with iPLEX gold chemistry (Agena Bioscience, San Diego, CA, USA) according to the protocol described. The Agena Bioscience TYPER software (version 4.0) was used to manage and analyze data.

Bioinformatics analysis

The online RegulomeDB software (<http://regulome.stanford.edu/>) and HaploReg software (<http://pubs.broadinstitute.org/mammals/haploreg/haploreg.php>) were used to predict the possible functional effects on these SNPs.

Statistical analysis

We used the Pearson's χ^2 test to analyze the differences in distribution of gender between the glioma patients and controls. The Student's t-test was used to evaluate differences in age between the case and control groups. We examined whether the genotype frequencies of SNPs in the control group was consistent with Hardy-Weinberg equilibrium (HWE) using χ^2 test. The association between *ANXA6* polymorphisms and glioma risk was assessed under the codominant, dominant, recessive and additive genetic models and haplotype analysis by PLINK software (version 1.07). Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to estimate the association between *ANXA6* polymorphisms and glioma risk with adjustment of gender and age using logistic regression analysis. We used the PLINK software and Haploview software (version 4.2) to assess the linkage disequilibrium (LD) between the each SNP. Survival distributions were estimated by using the Kaplan-Meier method and differences in the survival were tested using the log-rank test. The hazard ratio (HR) and 95% CI were calculated from the univariate Cox proportional hazards model to estimate the association between SNPs of *ANXA6* polymorphism with PFS and OS in glioma. Multivariate Cox models were performed to compute HR and 95% CI, after adjustment potential risk factors that could affect glioma outcome such as age, gender, and extent of resection. All *P* values were 2 sided and $P < 0.05$ was considered statistically significant. Statistical analysis was conducted using SPSS 20.0 statistical package (SPSS, Chicago, IL).

Results

Subject characteristics

We computed basic descriptive statistics for age, gender, WHO classification, WHO grade, surgical methods, chemotherapy, radiotherapy and survival condition (Table 1). The case-control study recruited 593 glioma patients (324 males and 269 females) with mean age 40.56 years old and 589 healthy controls (325 males and 264 females) with mean age 40.98 years old. The statistical analysis results showed that no statistically significant differences was found between glioma patients and controls with regarded to gender and age distribution ($p > 0.05$).

Details of the selected SNPs

The nine SNPs in the *ANXA6* gene were successfully genotyped (call rate $>95\%$). The details of the selected SNPs including chromosome id, position, role, allele and potential function predicted by the RegulomeDB software and HaploReg software about these variants are listed in Table 2. The results showed

that rs4958892 and rs3762993 are likely to affect expression quantitative trait loci (eQTL), TF binding, and DNase peak. The SNPs rs4346760 and rs9324677 are likely to affect TF binding, any motif and DNase peak. More detailed results are described in Table 2. The distributions of the nine *ANXA6* polymorphisms genotypes frequencies among the controls were in agreement with the HWE ($p > 0.05$), as shown in Table 2.

ANXA6 polymorphisms and glioma risk

The allele A frequency of rs3762993 in the patients with glioma was significantly different from those among the control subjects ($p = 0.007$) (Table 2). And the results found that individuals carrying the allele A frequency of rs3762993 was associated with significantly a 1.26-fold increased risk of glioma (OR = 1.26, 95% CI: 1.07–1.49). However, no significant association was found between the other eight polymorphisms *ANXA6* of and glioma risk in the allele model.

In order to explore the association between the nine polymorphisms of *ANXA6* and glioma risk, genetic models (codominant, dominant, recessive, and additive) by logistic regression analysis with adjustment for age and gender were applied (Table 3). The results showed that individuals with the AG (adjusted OR = 1.32, 95% CI: 1.03–1.69, $p = 0.029$) and AA genotypes of rs3762993 (adjusted OR = 1.52, 95% CI: 1.07–2.18, $p = 0.021$) were associated with increased risk of glioma compared with those with the GG genotype, respectively. Moreover, we also found that rs3762993 was associated with increased risk of glioma under the dominant model and the additive model, respectively (AA-AG vs. GG: adjusted OR = 1.36, 95% CI: 1.08–1.72, $p = 0.009$; adjusted OR = 1.26, 95% CI: 1.06–1.48, $p = 0.007$). No association was found between other eight SNPs of *ANXA6* and glioma risk (Table 3).

We used the PLINK software and Haploview software to assess the LD between the each SNP. The results found that a small haplotype block with strong LD between the two SNPs (rs78243462 and rs4346760), as shown in Figure 1. However, the haplotype analysis results showed that no significant association was found between these haplotypes and glioma risk (Table 4).

Statistical analysis results revealed that the SNP rs11960458 was remarkably an increased risk of glioma after adjusting for gender and age (allele C vs. T: OR = 1.29, 95% CI: 1.01–1.63, $p = 0.038$; CT vs. TT: OR = 1.55, 95% CI: 1.03–2.33, $p = 0.034$; CC vs. TT: OR = 1.79, 95% CI: 1.09–2.94, $p = 0.022$; CC-CT vs. TT: OR = 1.62, 95% CI: 1.1–2.37, $p = 0.014$; additive: OR = 1.35, 95% CI: 1.05–1.73, $p = 0.018$) in the age more than 41 years old (Table 5). The SNP rs4958892 was significantly associated with a decreased risk of glioma in the age more than 41 years old (AA vs. GG: OR = 0.58, 95% CI: 0.34–0.99, $p = 0.047$; additive: OR = 0.77, 95% CI: 0.60–0.99, $p = 0.041$) and males (AA vs. GG: OR = 0.61, 95% CI: 0.37–0.99, $p = 0.046$; additive: OR = 0.79, 95% CI: 0.63–0.98, $p = 0.036$) (Table 5).

Moreover, rs3762993 was found to be associated significantly associated an increased risk of glioma in the age more than 41 years old (AA-AG vs. GG: 1.42, 95% CI: 1.00–2.01, $p = 0.049$), and age $\leq$ 41 years old (A vs. G: OR = 1.28, 95% CI: 1.01–1.61, $p = 0.038$; AA vs. GG: OR = 1.88, 95% CI: 1.11–3.17, $p = 0.019$; AA vs. AG-GG: OR = 1.73, 95% CI: 1.06–2.82, $p = 0.029$; additive: OR = 1.31, 95% CI: 1.03–1.67, $p = 0.029$), females (A vs. G: OR = 1.47, 95% CI: 1.14–1.89, $p = 0.003$; AA vs. GG: OR = 1.60, 95% CI: 1.11–2.31, $p = 0.012$; AG vs. GG: OR = 2.11, 95% CI: 1.20–3.71, $p = 0.010$; AA vs. AG-GG: OR = 1.69, 95% CI: 1.19–2.40, $p = 0.003$; additive: OR = 1.50, 95% CI: 1.16–1.94, $p = 0.002$) and low grade glioma (A vs. G: OR = 1.31, 95% CI: 1.09–1.59, $p = 0.005$; AA vs. GG: OR = 1.38, 95% CI: 1.04–1.84, $p = 0.024$; AG vs. GG: OR = 1.65, 95% CI: 1.11–2.46, $p = 0.014$; AA vs. AG-GG: OR = 1.44, 95% CI: 1.11–1.88, $p = 0.007$; additive: OR = 1.31, 95% CI: 1.08–1.58, $p = 0.005$), as shown in Table 5.

Impact of clinical factors on glioma survival

In this study, we investigated the impact clinical factors, including gender, age, WHO grade, extent of resection, radiotherapy and chemotherapy, on the OS and PFS of glioma patients using the univariate and Cox regression analysis (Table 6, Figure 2). The results showed that the younger patients (age $<$ 40) had a longer OS than the older (age $\geq$ 40 years) (log-rank $p = 0.032$), and the age $\geq$ 40 was a hazardous factor with a 1.19-fold increased risk of death on OS in glioma patients, compared with the age $<$ 40 (HR = 1.19, 95% CI = 1.00–1.41, $p = 0.049$). The gross-total resection (GTR) of glioma patients had a longer OS (log-rank $p < 0.001$) and PS (log-rank $p < 0.001$) than the near-total resection (NTR) and sub-total resection (STR) of glioma patients, and had a better prognosis of glioma patients (OS: HR = 0.63, 95% CI: 0.52–0.76, $p < 0.001$, and PFS: HR = 0.59, 95% CI: 0.91–0.71, $p < 0.001$). Furthermore, we also found that the glioma patients with the chemotherapy treatment was also associated with a reduced risk of death on OS (log-rank $p < 0.001$, HR = 0.66, 95% CI: 0.55–0.78, $p < 0.001$) and PFS (log-rank $p = 0.007$, HR = 0.81, 95% CI: 0.67–0.96, $p = 0.017$), compared with the no chemotherapy treatment. However, no significant associations were found between the gender, WHO grade, radiotherapy and the prognosis of glioma patients as measured by OS and PFS.

Stratified analysis showed the patients with the GTR surgery was also decreased risk of death on OS in astrocytoma (OS: log-rank $p < 0.001$, HR = 0.67, 95% CI: 0.54–0.85, $p = 0.001$), high-grade glioma (OS: log-rank $p < 0.001$, HR = 0.62, 95% CI: 0.46–0.83, $p = 0.002$), and low grade glioma (OS: log-rank $p < 0.001$, HR = 0.67, 95% CI: 0.54–0.84, $p = 0.001$) (Table 6). In addition, the chemotherapy treatment was also positive factors in astrocytoma patients (OS: log-rank $p < 0.001$, HR = 0.64, 95% CI: 0.52–0.83, $p < 0.001$; PFS: log-rank $p = 0.001$, HR = 0.60, 95% CI: 0.18–0.76, $p < 0.001$), high-grade glioma patients (OS: log-rank $p < 0.001$, HR = 0.57, 95% CI: 0.42–0.78, $p < 0.001$; PFS: log-rank $p = 0.001$, HR = 0.55, 95% CI: 0.41–0.75, $p < 0.001$), and low grade glioma patients (OS: log-rank $p < 0.001$, HR = 0.66, 95% CI: 0.52–0.96, $p < 0.001$; PFS: log-rank $p = 0.001$, HR = 0.60, 95% CI: 0.48–0.76, $p < 0.001$) (Table 6).

Impact of SNPs on glioma survival

We also evaluated the effect of the nine *ANXA6* polymorphisms on the glioma patients with OS and PFS using the log-rank tests and Cox regression analysis. Interestingly, we found the CC genotype of rs888988 was significantly correlated with poor prognosis in glioma patients on OS (log-rank $p = 0.026$,

HR = 1.79, 95% CI: 1.05–3.05, $p = 0.033$) and PFS (log-rank $p = 0.018$, HR = 1.97, 95% CI: 1.14–3.41, $p = 0.016$) compared to the GG genotype (Table 7, Figure 3). The CG genotype of rs888988 was also found to have a negative effect on OS (HR = 1.80, 95% CI: 1.05–3.07, $p = 0.031$) and PFS (HR = 1.99, 95% CI: 1.15–3.45, $p = 0.014$) compared to the common GG genotype. However, no significant associations were identified between the other eight SNPs and OS or PFS of glioma patients. The multivariate Cox regression analysis did not find the effects of the nine SNPs in the *ANXA6* gene on the prognosis of glioma patients.

Stratified analysis revealed that the TC genotype of rs11960458 was significantly associated with a decreased survival of the low grade glioma patients as measured by OS, compared with the CC genotype carriers (HR = 1.44, 95% CI: 1.04–1.98, $p = 0.027$) (Table 7, Figure 3). We also found the CC genotype of rs888988 was associated with an increase in mortality hazard of borderline statistical significance as measured by OS and PFS of age more than 40 years old glioma patients (OS: log-rank $p = 0.034$, HR = 2.28, 95% CI: 1.12–4.63, $p = 0.022$; PFS: log-rank $p = 0.026$, HR = 2.30, 95% CI: 1.13–4.66, $p = 0.021$) and male glioma patients (OS: log-rank $p = 0.016$, HR = 2.67, 95% CI: 1.18–6.02, $p = 0.018$; PFS: log-rank $p = 0.013$, HR = 2.61, 95% CI: 1.16–5.9, $p = 0.021$) compared to the GG genotype. Furthermore, the GC genotype of rs888988 was also found to have a negative effect on OS and PFS in the age more than 40 years old glioma patients (OS: HR = 2.22, 95% CI: 1.07–4.61, $p = 0.032$; PFS: HR = 2.20, 95% CI: 1.06–4.55, $p = 0.034$) and male glioma patients (OS: HR = 2.93, 95% CI: 1.27–6.79, $p = 0.012$; PFS: HR = 2.94, 95% CI: 1.27–6.81, $p = 0.012$) compared to the common GG genotype.

Discussion

This study was firstly to investigate the associations between *ANXA6* polymorphisms and glioma risk and prognosis of glioma patients in the Chinese Han population. Our results indicated that the three SNPs (rs11960458, rs4958892, and rs3762993) of *ANXA6* were significantly associated with the risk of glioma. Interestingly, rs11960458 and rs888988 were found to be correlated with poor prognosis of glioma patients. Furthermore, those clinical factors including age, extent of resection and chemotherapy were found to be key prognostic factors in survival of glioma patients.

The *ANXA6* gene is approximately 60 kbp long and contains 26 exons. It encodes a protein (Annexin A6) of about 68 kDa that consists of eight 68-amino acid repeats separated by linking sequences of variable lengths. Annexin A6 belongs to a family of Ca^{2+} -dependent membrane and phospholipid binding proteins. More recently, studies identified that AnxA6 involved in multiple avenues of cell motility [10]. AnxA6 is closely associated with a variety of tumors, which exhibits dual functions in cancer, acting either as a tumor suppressor or promoter. It has been implicated that AnxA6 is a marker for squamous cervical cancer development [21]. A study by Wang et al demonstrated that the downregulation of AnxA6 by yin yang 1-induced promoter methylation of *ANXA6* CpG is found in gastric cancer cells enhanced gastric cancer invasiveness and progression through interrupted activity of Ras/MAPK signaling [22]. Koese et al [23] demonstrated that AnxA6 displays tumor suppression effects on A431 cells by facilitating the membrane targeting of PKC α and/or p120GAP, which is mediated by downregulation of the EGF-induced Ras activity and reduced expression levels of cyclin D1. AnxA6 participates in enhancing the localization and stabilization of activated EGFR on cell surfaces, and/or enhancing the localization of focal adhesions. The consequently enhanced activation of FAK triggers a sustained activation of downstream effectors that promote the motility and invasiveness of BT-549 BC cells [24].

The three SNPs (rs11960458, rs4958892, and rs3762993) of *ANXA6* were found to be significantly associated with the risk of glioma; rs11960458 and rs888988 were found to be correlated with poor prognosis of glioma patients in this study. The 52 SNPs from three genes of the *ANXA* family were selected from public databases and genotyped in 443 osteonecrosis of the femoral head patients and 273 control subjects using the Affymetrix Targeted Genotyping 3 K Chip array, results found that SNPs rs11960458 and rs9324677 of the *ANXA6* gene were significantly associated with the risk of osteonecrosis of the femoral head in the Korean population, but no association was found between rs4958892 and rs888988 in *ANXA6* and osteonecrosis of the femoral head risk [25]. This study was firstly to investigate the associations between *ANXA6* polymorphisms and glioma risk and prognosis of glioma patients in the Chinese Han population. Therefore, we need to confirm the conclusion of this study in multi-ethnic population with larger scale.

In this study, those clinical factors including age, extent of resection and chemotherapy were found to be key prognostic factors in survival of glioma patients. The comprehensive analysis of multicentric glioma patients revealed that age younger than 54 years, surgical resection, and radiotherapy were significantly associated with improved survival and were independent prognostic factors for OS [26]. Gui et al [27] also demonstrated that age, surgical resection and chemotherapy influenced the survival rates of glioma patients. A systematic review and meta-analysis study showed that GTR substantially improves overall and progression-free survival compared with STR [28]. Our results did not find any significant association between radiotherapy and prognosis of glioma patients. However, Keime-Guibert et al [29] study indicated that radiotherapy resulted in a modest improvement in survival in elderly patients with glioblastoma. Therefore, we need to confirm the results in the further study with a large sample.

Several limitations should be addressed in this study. The sample in this study limited to Chinese population, therefore, the conclusion of this study warrants further confirmation in multi-ethnic population with larger scale. Additionally, this study did not elucidate the molecular mechanism under which the *ANXA6* polymorphisms affect the glioma risk and the prognosis in glioma patients. Further functional studies will be required to elucidate these specific mechanisms of the *ANXA6* polymorphisms affect the prognosis in glioma patient.

In conclusion, the results indicated that age, extent of resection and chemotherapy were found to be key prognostic factors in survival of glioma patients. Moreover, the present study is the first time to find that *ANXA6* polymorphisms are associated with the glioma risk and affect the progression of glioma patients. These observations may be potentially valuable prognostic markers for glioma patients.

Abbreviations

CNS: central nervous system; 95% CI: 95% confidence interval; EDTA: ethylene diamine tetraacetic acid; HR: hazard ratio; HWE: Hardy-Weinberg equilibrium; LD: linkage disequilibrium; MAF: minor allele frequency; OR: odds ratio; OS: overall survival; PFS: progress-free survival; SNPs: single-nucleotide

Declarations

Ethics approval and consent to participate

The protocol of this study was approved by the Ethics Committee of Tangdu Hospital and was conducted in accordance with the principles of the Declaration of Helsinki. All participants gave written informed consent prior to blood samples collection in the study.

Consent for publication

Not applicable.

Competing interests

The authors declare that there are no conflicts of interest.

Funding

Not applicable.

Authors' contributions

Yuan Shao contributed to the design of the study and performed the statistical analysis. Tuo Wang was responsible for manuscript preparation. Yao Sun and Zichao Xiong performed the experiments. Jiamin Wu and Xiaoying Ding contributed to collect the data. Xiaoye Guo was responsible for checking the data. All authors were responsible for drafting the manuscript, read and approved the final version.

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Tables

Table 1 Characteristics of glioma patients and healthy controls

Characteristics		Cases (%)	Controls (%)	<i>p</i>
Total		593	589	
Age, Mean ± SD (year)		40.56±18.11	40.98±11.96	0.640
Gender	Male	324 (54.6)	325 (55.2)	0.852
	Female	269 (45.5)	264 (44.8)	
WHO classification	Astrocytoma	366 (61.7)		
	Ependymoma	38 (6.4)		
	Glioblastoma	39 (6.6)		
	Oligodendrocytes astrocytoma	94 (15.9)		
	Oligodendroglioma	19 (3.2)		
	Others	37 (6.2)		
WHO grade	I-II	379 (63.9)		
	III-IV	214 (36.1)		
Surgical method	STR + NTR	185 (31.2)		
	GTR	408 (68.8)		
Chemotherapy	No	348 (58.7)		
	Platinum	121 (20.4)		
	Nimustine	72 (12.1)		
	Temozolomide	52 (8.8)		
Radiotherapy	No	58 (9.8)		
	Conformal radiotherapy	159 (26.8)		
	Gamma knife	376 (63.4)		
Survival condition	Survival	41 (6.9)		
	Death	528 (89.0)		
	Lost	24 (4.1)		

WHO: World Health Organization; GTR: gross-total resection; NTR: near-total resection; STR: sub-total resection

p < 0.05 indicates statistical significance.

Table 2 The information of SNPs in *ANXA6* and association with glioma risk

SNP-ID	Chr: Position	Role	Allele A/B	Regulome DB Score	Haploreg	HWE- <i>p</i>	MAF	MAF	OR (95% CI)	<i>p</i>
							Case	Control		
rs11960458	5:151100959	3'- UTR	C/T	4	Promoter histone marks, enhancer histone marks, DNase, proteins bound, motifs changed, selected eQTL hits	1.000	0.47	0.451	1.08 (0.92- 1.27)	0.353
rs4958892	5:151103534	Intron	A/G	1f	Enhancer histone marks, motifs changed, selected eQTL hits	1.000	0.332	0.369	0.85 (0.72- 1.01)	0.061
rs78243462	5:151111165	Intron	C/T	4	Promoter histone marks, enhancer histone marks, DNase, proteins bound, motifs changed	0.495	0.066	0.063	1.05 (0.75- 1.45)	0.787
rs4346760	5:151113909	Intron	A/C	3a	Enhancer histone marks, DNase, proteins bound, motifs changed	0.162	0.489	0.493	0.98 (0.84- 1.16)	0.838
rs4958897	5:151120172	Intron	C/T	5	Enhancer histone marks, DNase, motifs changed, selected eQTL hits	0.303	0.413	0.398	1.07 (0.90- 1.26)	0.452
rs3762993	5:151130672	Intron	A/G	1f	Enhancer histone marks, DNase, motifs changed	0.356	0.390	0.337	1.26 (1.07- 1.49)	0.007
rs9324677	5:151134177	Intron	A/C	3a	Enhancer histone marks, motifs changed, selected eQTL hits	0.866	0.423	0.427	0.98 (0.84- 1.16)	0.850
rs13185706	5:151142998	Intron	A/C	5	Enhancer histone marks, motifs changed, selected eQTL hits	0.648	0.101	0.101	1.00 (0.77- 1.31)	0.999
rs888988	5:151143209	Intron	C/G	5	Promoter histone marks, enhancer histone marks	0.274	0.165	0.185	0.87 (0.71- 1.08)	0.218

SNP: single nucleotide polymorphism; Chr: chromosome; HWE: Hardy-Weinberg equilibrium; Regulome DB Score: 1f: eQTL + TF binding / DNase peak; 3a: TF binding + any motif + DNase peak; 4: TF binding + DNase peak; 5: TF binding or DNase peak; eQTL: expression quantitative trait loci; MAF: minor allele frequency; OR: odds ratio; CI: confidence interval. *p* < 0.05 indicates statistical significance.

Table 3 Genetics models analyses of association polymorphisms of *ANXA6* with glioma risk

SNP-ID	Model	Genotype	Case	Control	OR (95%CI)	<i>p</i>	Adjust OR (95%CI)	<i>p</i>
rs11960458	Codominant	TT	163 (27.5)	176 (30.0)	1.00		1.00	
		CT	301 (50.8)	291 (49.7)	1.12 (0.86-1.46)	0.417	1.12 (0.86-1.47)	0.402
		CC	128 (21.6)	119 (20.3)	1.16 (0.84-1.61)	0.372	1.16 (0.84-1.62)	0.364
	Dominant	TT	163 (27.5)	176 (30.0)	1.00		1.00	
		CC-CT	429 (72.5)	410 (70.0)	1.13 (0.88-1.45)	0.343	1.13 (0.88-1.46)	0.330
	Recessive	CT-TT	464 (78.4)	467 (79.7)	1.00		1.00	
		CC	128 (21.6)	119 (20.3)	1.08 (0.82-1.43)	0.580	1.08 (0.82-1.43)	0.579
	Additive	---	---	---	1.08 (0.92-1.27)	0.351	1.08 (0.92-1.27)	0.342
rs4958892	Codominant	GG	265 (44.8)	235 (39.8)	1.00		1.00	
		AG	261 (44.1)	275 (46.6)	0.84 (0.66-1.07)	0.166	0.84 (0.66-1.07)	0.160
		AA	66 (11.1)	80 (13.6)	0.73 (0.51-1.06)	0.098	0.73 (0.51-1.06)	0.099
	Dominant	GG	265 (44.8)	235 (39.8)	1.00		1.00	
		AA-AG	327 (55.2)	355 (60.2)	0.82 (0.65-1.03)	0.086	0.82 (0.65-1.03)	0.083
	Recessive	AG-GG	526 (88.9)	510 (86.4)	1.00		1.00	
		AA	66 (11.1)	80 (13.6)	0.80 (0.56-1.13)	0.209	0.80 (0.57-1.14)	0.212
	Additive	---	---	---	0.85 (0.72-1.01)	0.062	0.85 (0.72-1.01)	0.061
rs78243462	Codominant	TT	517 (87.2)	516 (87.9)	1.00		1.00	
		CT	74 (12.5)	68 (11.6)	1.09 (0.76-1.54)	0.645	1.08 (0.76-1.54)	0.667
		CC	2 (0.3)	3 (0.5)	0.67 (0.11-4.00)	0.656	0.67 (0.11-4.06)	0.666
	Dominant	TT	517 (87.2)	516 (87.9)	1.00		1.00	
		CC-CT	76 (12.8)	71 (12.1)	1.07 (0.76-1.51)	0.708	1.06 (0.75-1.50)	0.729
	Recessive	CT-TT	591 (99.7)	584 (99.5)	1.00		1.00	
		CC	2 (0.3)	3 (0.5)	0.66 (0.11-3.96)	0.648	0.67 (0.11-4.02)	0.659
	Additive	---	---	---	1.05 (0.75-1.45)	0.787	1.04 (0.75-1.45)	0.805
rs4346760	Codominant	CC	159 (26.9)	160 (27.1)	1.00		1.00	
		AC	287 (48.5)	278 (47.1)	1.04 (0.79-1.37)	0.786	1.04 (0.79-1.37)	0.788
		AA	146 (24.7)	152 (25.8)	0.97 (0.70-1.33)	0.833	0.97 (0.71-1.33)	0.842
	Dominant	CC	159 (26.9)	160 (27.1)	1.00		1.00	
		AA-AC	433 (73.1)	430 (72.9)	1.01 (0.78-1.31)	0.920	1.01 (0.78-1.31)	0.918
	Recessive	AC-CC	446 (75.3)	438 (74.2)	1.00		1.00	
		AA	146 (24.7)	152 (25.8)	0.94 (0.73-1.23)	0.663	0.95 (0.73-1.23)	0.676
	Additive	---	---	---	0.98 (0.84-1.15)	0.842	0.99 (0.84-1.15)	0.852
rs4958897	Codominant	TT	208 (35.1)	219 (37.2)	1.00		1.00	
		CT	280 (47.2)	270 (45.9)	1.09 (0.85-1.41)	0.496	1.09 (0.84-1.40)	0.513
		CC	105 (17.7)	99 (16.8)	1.12 (0.80-1.56)	0.517	1.11 (0.80-1.56)	0.526
	Dominant	TT	208 (35.1)	219 (37.2)	1.00		1.00	
		CC-CT	385 (64.9)	369 (62.8)	1.10 (0.87-1.39)	0.438	1.10 (0.86-1.39)	0.454
	Recessive	CT-TT	488 (82.3)	489 (83.2)	1.00		1.00	
		CC	105 (17.7)	99 (16.8)	1.06 (0.79-1.44)	0.693	1.06 (0.79-1.44)	0.696
	Additive	---	---	---	1.06 (0.90-1.25)	0.460	1.06 (0.90-1.25)	0.472
rs3762993	Codominant	GG	222 (37.4)	264 (44.9)	1.00		1.00	
		AG	279 (47.0)	252 (42.9)	1.32 (1.03-1.69)	0.029	1.32 (1.03-1.69)	0.029
		AA	92 (15.5)	72 (12.2)	1.52 (1.06-2.17)	0.021	1.52 (1.07-2.18)	0.021
	Dominant	GG	222 (37.4)	264 (44.9)	1.00		1.00	
		AA-AG	371 (62.6)	324 (55.1)	1.36 (1.08-1.72)	0.009	1.36 (1.08-1.72)	0.009
	Recessive	AG-GG	501 (84.5)	516 (87.8)	1.00		1.00	
		AA	92 (15.5)	72 (12.2)	1.32 (0.94-1.83)	0.105	1.32 (0.95-1.84)	0.102
	Additive	---	---	---	1.25 (1.06-1.48)	0.008	1.26 (1.06-1.48)	0.007
rs9324677	Codominant	CC	195 (33.1)	194 (33.1)	1.00		1.00	
		AC	290 (49.2)	284 (48.5)	1.02 (0.79-1.31)	0.905	1.02 (0.79-1.32)	0.895
		AA	104 (17.7)	108 (18.4)	0.96 (0.69-1.34)	0.802	0.96 (0.69-1.35)	0.820
	Dominant	CC	195 (33.1)	194 (33.1)	1.00		1.00	
		AA-AC	394 (66.9)	392 (66.9)	1.00 (0.78-1.28)	1.000	1.00 (0.79-1.28)	0.986
	Recessive	AC-CC	485 (82.3)	478 (81.6)	1.00		1.00	
		AA	104 (17.7)	108 (18.4)	0.95 (0.70-1.28)	0.731	0.95 (0.71-1.28)	0.746
	Additive	---	---	---	0.98 (0.84-1.16)	0.850	0.99 (0.84-1.16)	0.868
rs13185706	Codominant	CC	481 (81.1)	476 (81.0)	1.00		1.00	
		AC	104 (17.5)	105 (17.9)	0.98 (0.73-1.32)	0.896	0.98 (0.73-1.32)	0.896
		AA	8 (1.3)	7 (1.2)	1.13 (0.41-3.14)	0.813	1.14 (0.41-3.16)	0.809
	Dominant	CC	481 (81.1)	476 (81.0)	1.00		1.00	
		AA-AC	112 (18.9)	112 (19.0)	0.99 (0.74-1.32)	0.944	0.99 (0.74-1.32)	0.945
	Recessive	AC-CC	585 (98.7)	581 (98.8)	1.00		1.00	
		AA	8 (1.3)	7 (1.2)	1.14 (0.41-3.15)	0.808	1.14 (0.41-3.16)	0.803
	Additive	---	---	---	1.00 (0.77-1.30)	0.999	1.00 (0.77-1.30)	0.998
rs888988	Codominant	GG	416 (70.2)	395 (67.2)	1.00		1.00	
		CG	158 (26.6)	169 (28.7)	0.89 (0.69-1.15)	0.364	0.88 (0.68-1.14)	0.350
		CC	19 (3.2)	24 (4.1)	0.75 (0.41-1.39)	0.365	0.75 (0.41-1.40)	0.367

Dominant	GG	416 (70.2)	395 (67.2)	1.00	1.00		
	CC-CG	177 (29.8)	193 (32.8)	0.87 (0.68–1.11)	0.271	0.87 (0.68–1.11)	0.261
Recessive	CG-GG	574 (96.8)	564 (95.9)	1.00	1.00		
	CC	19 (3.2)	24 (4.1)	0.78 (0.42–1.44)	0.422	0.78 (0.42–1.44)	0.426
Additive	---	---	---	0.88 (0.71–1.08)	0.227	0.88 (0.71–1.08)	0.221

SNP: single nucleotide polymorphism; OR: odds ratio; CI: confidence interval.

Adjust OR (95%CI) were calculated by logistic regression analysis with adjustments for age and gender.

$p < 0.05$ indicates statistical significance.

Table 4 Association haplotype with glioma risk

SNP-ID	Haplotype	Case	Control	adjust OR (95%CI)	p
rs78243462 rs4346760	TA	0.9341	0.9369	0.96 (0.69–1.33)	0.804
	CA	0.5769	0.5725	1.02 (0.86–1.19)	0.859
	CC	0.511	0.5094	1.01 (0.86–1.18)	0.955

SNP: single nucleotide polymorphism; OR: odds ratio; CI: confidence interval.

Adjust OR (95%CI) was calculated by logistic regression analysis with adjustments for age and gender.

$p < 0.05$ indicates statistical significance.

Table 5 Association of ANXA6 polymorphisms with glioma risk stratified by age, gender and WHO grade

SNP-ID	Model	Genotype	Case	Control	OR (95% CI)	<i>p</i>	Case	Control	OR (95% CI)	<i>p</i>
Age			>41				≤ 41			
rs11960458	Allele	T	297 (50.3)	293 (56.6)	1.00		330 (55.6)	350 (53.5)	1.00	
		C	293 (49.7)	225 (43.4)	1.29 (1.01-1.63)	0.038	264 (44.4)	304 (46.5)	0.92 (0.74-1.15)	0.470
	Codominant	TT	70 (23.7)	84 (32.4)	1.00		93 (31.3)	92 (28.1)	1.00	
		CT	157 (53.2)	125 (48.3)	1.55 (1.03-2.33)	0.034	144 (48.5)	166 (50.8)	0.93 (0.63-1.37)	0.728
	Dominant	CC	68 (23.1)	50 (19.3)	1.79 (1.09-2.94)	0.022	60 (20.2)	69 (21.1)	0.91 (0.57-1.46)	0.694
		TT	70 (23.7)	84 (32.4)	1.00		93 (31.3)	92 (28.1)	1.00	
	Recessive	CC-CT	225 (76.3)	175 (67.6)	1.62 (1.1-2.37)	0.014	204 (68.7)	235 (71.9)	0.93 (0.64-1.33)	0.681
		CT-TT	227 (76.9)	209 (80.7)	1.00		237 (79.8)	258 (78.9)	1.00	
	Additive	CC	68 (23.1)	50 (19.3)	1.34 (0.88-2.05)	0.169	60 (20.2)	69 (21.1)	0.95 (0.63-1.43)	0.803
		---	---	---	1.35 (1.05-1.73)	0.018	---	---	0.95 (0.75-1.21)	0.681
rs4958892	Allele	G	406 (68.8)	331 (63.4)	1.00		385 (64.8)	414 (62.9)	1.00	
		A	184 (31.2)	191 (36.6)	0.79 (0.61-1.01)	0.057	209 (35.2)	244 (37.1)	0.92 (0.73-1.16)	0.486
	Codominant	GG	144 (48.8)	110 (42.1)	1.00		121 (40.7)	125 (38.0)	1.00	
		AG	118 (40)	111 (42.5)	0.81 (0.56-1.16)	0.248	143 (48.1)	164 (49.8)	0.96 (0.68-1.37)	0.841
	Dominant	AA	33 (11.2)	40 (15.3)	0.58 (0.34-0.99)	0.047	33 (11.1)	40 (12.2)	0.81 (0.46-1.42)	0.464
		GG	144 (48.8)	110 (42.1)	1.00		121 (40.7)	125 (38.0)	1.00	
	Recessive	AA-AG	151 (51.2)	151 (57.9)	0.74 (0.53-1.05)	0.092	176 (59.3)	204 (62.0)	0.93 (0.67-1.31)	0.692
		AG-GG	262 (88.8)	221 (84.7)	1.00		264 (88.9)	289 (87.8)	1.00	
	Additive	AA	33 (11.2)	40 (15.3)	0.64 (0.39-1.07)	0.088	33 (11.1)	40 (12.2)	0.83 (0.49-1.40)	0.480
		---	---	---	0.77 (0.6-0.99)	0.041	---	---	0.92 (0.71-1.19)	0.524
rs3762993	Allele	G	359 (60.6)	341 (65.6)	1.00		364 (61.3)	439 (66.9)	1.00	
		A	233 (39.4)	179 (34.4)	1.24 (0.97-1.58)	0.089	230 (38.7)	217 (33.1)	1.28 (1.01-1.61)	0.038
	Codominant	GG	108 (36.5)	117 (45.0)	1.00		114 (38.4)	147 (44.8)	1.00	
		AG	143 (48.3)	107 (41.2)	1.43 (0.99-2.07)	0.058	136 (45.8)	145 (44.2)	1.17 (0.82-1.67)	0.390
	Dominant	AA	45 (15.2)	36 (13.8)	1.39 (0.82-2.33)	0.220	47 (15.8)	36 (11.0)	1.88 (1.11-3.17)	0.019
		GG	108 (36.5)	117 (45.0)	1.00		114 (38.4)	147 (44.8)	1.00	
	Recessive	AA-AG	188 (63.5)	143 (55.0)	1.42 (1-2.01)	0.049	183 (61.6)	181 (55.2)	1.3 (0.93-1.82)	0.125
		AG-GG	251 (84.8)	224 (86.2)	1.00		250 (84.2)	292 (89.0)	1.00	
	Additive	AA	45 (15.2)	36 (13.8)	1.15 (0.71-1.87)	0.572	47 (15.8)	36 (11.0)	1.73 (1.06-2.82)	0.029
		---	---	---	1.23 (0.97-1.58)	0.094	---	---	1.31 (1.03-1.67)	0.029
Gender			Male				Female			
rs4958892	Allele	G	439 (67.3)	350 (53.8)	1.00		352 (66.2)	344 (64.9)	1.00	
		A	213 (32.7)	304 (46.8)	0.78 (0.62-0.98)	0.033	180 (33.8)	186 (35.1)	0.95 (0.73-1.22)	0.666
	Codominant	GG	149 (45.7)	92 (28.3)	1.00		116 (43.6)	109 (41.1)	1.00	
		AG	141 (43.3)	166 (51.1)	0.80(0.58-1.12)	0.193	120 (45.1)	126 (47.5)	0.88 (0.61-1.26)	0.479
	Dominant	AA	36 (11)	69 (21.2)	0.61 (0.37-0.99)	0.046	30 (11.3)	30 (11.3)	0.94 (0.53-1.66)	0.824
		GG	149 (45.7)	92 (28.3)	1.00		116 (43.6)	109 (41.1)	1.00	
	Recessive	AA-AG	177 (54.3)	235 (72.3)	0.75 (0.55-1.03)	0.076	150 (56.4)	156 (58.9)	0.89 (0.63-1.26)	0.503
		AG-GG	290 (89)	258 (79.4)	1.00		236 (88.7)	235 (88.7)	1.00	
	Additive	AA	36 (11)	69 (21.2)	0.68 (0.43-1.08)	0.100	30 (11.3)	30 (11.3)	1.00 (0.59-1.72)	0.990
		---	---	---	0.79 (0.63-0.98)	0.036	---	---	0.94 (0.72-1.21)	0.623
rs3762993	Allele	G	403 (61.8)	414 (63.9)	1.00		320 (59.9)	363 (68.8)	1.00	
		A	249 (38.2)	244 (37.7)	1.12 (0.89-1.40)	0.342	214 (40.1)	165 (31.3)	1.47 (1.14-1.89)	0.003
	Codominant	GG	129 (39.6)	125 (38.6)	1.00		93 (34.8)	125 (47.3)	1.00	
		AG	145 (44.5)	164 (50.6)	1.12 (0.80-1.57)	0.496	134 (50.2)	113 (42.8)	1.60 (1.11-2.31)	0.012
	Dominant	AA	52 (16)	40 (12.3)	1.22 (0.76-1.93)	0.408	40 (15)	26 (9.8)	2.11 (1.20-3.71)	0.010
		GG	129 (39.6)	125 (38.6)	1.00		93 (34.8)	125 (47.3)	1.00	
	Recessive	AA-AG	197 (60.4)	204 (63.0)	1.15 (0.84-1.57)	0.392	174 (65.2)	139 (52.7)	1.69 (1.19-2.40)	0.003
		AG-GG	274 (84)	289 (89.2)	1.00		227 (85)	238 (90.2)	1.00	
	Additive	AA	52 (16)	40 (12.3)	1.15 (0.74-1.76)	0.536	40 (15)	26 (9.8)	1.64 (0.97-2.79)	0.066
		---	---	---	1.11 (0.89-1.38)	0.361	---	---	1.50 (1.16-1.94)	0.002
WHO grade			I-II				III-IV			
rs3762993	Allele	G	455 (60)	780 (66.3)	1.00		268 (62.6)	780 (66.3)	1.00	
		A	303 (40)	396 (33.7)	1.31 (1.09-1.59)	0.005	160 (37.4)	396 (33.7)	1.18 (0.93-1.48)	0.167
	Codominant	GG	137 (36.1)	264 (44.9)	1.00		85 (39.7)	264 (44.9)	1.00	
		AG	181 (47.8)	252 (42.9)	1.38 (1.04-1.84)	0.024	98 (45.8)	252 (42.9)	1.20 (0.85-1.68)	0.303
	Dominant	AA	61 (16.1)	72 (12.2)	1.65 (1.11-2.46)	0.014	31 (14.5)	72 (12.2)	1.29 (0.79-2.10)	0.314
		GG	137 (36.1)	264 (44.9)	1.00		85 (39.7)	264 (44.9)	1.00	
	Recessive	AA-AG	242 (63.9)	324 (55.1)	1.44 (1.11-1.88)	0.007	129 (60.3)	324 (55.1)	1.22 (0.88-1.67)	0.231
		AG-GG	318 (83.9)	516 (87.8)	1.00		183 (85.5)	516 (87.8)	1.00	
	Additive	AA	61 (16.1)	72 (12.2)	1.39 (0.96-2.01)	0.082	31 (14.5)	72 (12.2)	1.17 (0.74-1.85)	0.492
		---	---	---	1.31 (1.08-1.58)	0.005	---	---	1.15 (0.92-1.44)	0.230

SNP: single nucleotide polymorphism; OR: odds ratio; CI: confidence interval.

OR (95% CI) were calculated by logistic regression analysis with adjustments for age and gender.

p < 0.05 indicates statistical significance.

Table 6 Univariate analysis of the impact of clinical factors on glioma patient OS and PFS

Group	Variable	Classification	No. of patients/events	1 / 3(year) ST %	MST (month)	OS Log-rank <i>p</i>	HR (95%CI)	<i>p</i>	1 / 3(year) ST %	MST (month)	PFS Log-rank <i>p</i>	HR(95%CI)	<i>p</i>
overall	Gender	Male	324/287	32.9/8.8	11		1.00		20.0/9.6	8		1.00	
		Female	269/241	31.6/9.2	11	0.458	1.06 (0.89-1.26)	0.496	15.3/8.8	8	0.272	1.09 (0.92-1.29)	0.324
	Age(years)	<40	262/224	36.1/12.1	12		1.00		20.5/11.8	8		1.00	
		≥40	331/304	29.3/6.6	11	0.032	1.19 (1.00-1.41)	0.049	15.8/7.1	8	0.081	1.15 (0.97-1.37)	0.118
	WHO grade	I	379/331	33.1/10.8	12		1.00		19.1/10.7	8		1.00	
		II	214/197	30.8/6.4	10	0.102	1.15 (0.96-1.37)	0.133	15.6/6.6	8	0.092	1.15 (0.96-1.37)	0.131
	Extent of resection	STR+ NTR	185/182	20.0/1.6	12		1.00		1.6/-	8		1.00	
		GTR	408/346	37.9/12.4	11	<0.001	0.63 (0.52-0.76)	<0.001	25.3/12.5	8	<0.001	0.59 (0.19-0.71)	<0.001
	Radiotherapy	No	58/47	43.1/-	8		1.00		19.0/-	6		1.00	
		CRT	159/131	24.8/14.3	10		0.87 (0.64-1.18)	0.362	21.6/15.3	8		1.06 (0.93-1.20)	0.402
		Gamma knife	376/350	33.8/6.2	11	0.562	0.95 (0.77-1.16)	0.599	16.3/5.8	8	0.244	-	
	Chemotherapy	No	348/326	26.7/-2.8	9		1.00		16.4/5.7	7		1.00	
		Yes	245/202	40.3/15.6	12	<0.001	0.66 (0.55-0.78)	<0.001	20.0/15.2	8	0.007	0.81 (0.67-0.96)	0.017
	Extent of resection	STR + NTR	116/114	20.7/-	9		1.00		1.7/-	7		1.00	
		GTR	250/214	37.2/10.6	11	<0.001	0.64 (0.52-0.83)	<0.001	24.7/11.7	8	0.001	0.60 (0.18-0.76)	<0.001
	Chemotherapy	No	219/204	27.4/-	10		1.00		16.9/5.8	7		1.00	
		Yes	147/124	38.8/13	12	<0.001	0.67 (0.54-0.85)	0.001	18/13.1	8	0.070	0.83 (0.66-1.04)	0.105
III-IV	Extent of resection	STR + NTR	65/64	16.9/-	9		1.00		1.5/-	7		1.00	
		GTR	149/133	36.9/8.3	11	<0.001	0.57 (0.42-0.78)	<0.001	21.9/8.8	8	0.001	0.55 (0.41-0.75)	<0.001
	Chemotherapy	No	125/121	23.2/-	9		1.00		14.4/-	7		1.00	
		Yes	89/76	41.6/12.5	12	<0.001	0.62 (0.46-0.83)	0.002	17.4/12	8	0.103	0.81 (0.60-1.08)	0.146
I-II	Extent of resection	STR + NTR	120/118	21.7/-	12		1.00		1.7/-	8		1.00	
		GTR	259/213	38.5/15.1	11	<0.001	0.66 (0.52-0.83)	<0.001	27.3/15	8	0.001	0.60 (0.48-0.76)	<0.001
	Chemotherapy	No	223/205	28.7/3.6	11		1.00		17.5/6.8	8		1.00	
		Yes	156/126	39.5/17.8	12	<0.001	0.67 (0.54-0.84)	0.001	21.6/17.4	8	0.034	0.80 (0.64-1.00)	0.057

WHO: World Health Organization; GTR: gross-total resection; NTR: near-total resection; STR: sub-total resection; OS: overall survival; PFS: progression free survival; ST: survival rate; MST: median survival time

HR: hazard ratio; 95% CI: 95% confidence interval

p < 0.05 indicates statistical significance.

Table 7 Univariate analysis of the association between ANXA6 polymorphisms and glioma patient OS and PFS

Group	SNP-ID	Genotype	No. of patients/events	1 / 3(year) ST %	MST (month)	OS Log- rank <i>p</i>	HR (95%CI)	<i>p</i>	1 / 3(year) ST %	MST (month)	PFS Log- rank <i>p</i>	HR (95%CI)	<i>p</i>
Overall	rs11960458	C/C	163/147	32.5/7.9	11		1.00		14.8/6.4	8		1.00	
		T/C	301/270	31.4/8.4	11		1.17 (0.91-1.50)	0.213	16.9/8.1	8		1.16 (0.91-1.49)	0.228
		T/T	128/110	33.6/10.8	12	0.282	1.17 (0.94-1.46)	0.168	22.7/11.5	8	0.250	1.18 (0.94-1.47)	0.148
	rs4958892	G/G	265/235	29.4/9.0	11		1.00		17/7.6	8		1.00	
		A/G	262/235	32.3/8.3	11		1.15 (0.87-1.54)	0.328	17/7.8	8		1.09 (0.82-1.46)	0.549
		A/A	66/58	43.9/10.4	12	0.330	1.22 (0.92-1.63)	0.176	21.2/11.1	8	0.651	1.13 (0.85-1.51)	0.410
	rs78243462	C/C	516/456	32.3/8.9	11		1.00		18.4/8.0	8		1.00	
		T/C	75/70	32.0/7.0	10		1.27 (0.32-5.08)	0.739	14.7/5.0	8		1.09 (0.27-4.36)	0.907
		T/T	2/2	50.0/-	12	0.561	1.43 (0.35-5.81)	0.622	/-	8	0.378	1.28 (0.31-5.20)	0.735
	rs4346760	C/C	159/136	33.7/12.9	11		1.00		17.8/12.1	8		1.00	
		A/C	288/263	34.4/6.9	12		0.90 (0.71-1.14)	0.386	16.5/6.4	8		0.89 (0.70-1.13)	0.338
		A/A	146/129	26.7/6.4	11	0.609	0.97 (0.79-1.20)	0.783	16.4/6.9	8	0.500	0.98 (0.79-1.21)	0.839
	rs4958897	C/C	106/96	30.2/9.0	11		1.00		17.0/8.2	8		1.00	
		C/T	279/243	31.8/10.8	11		1.00 (0.78-1.28)	0.987	16.3/9.3	8		0.99 (0.77-1.26)	0.914
		T/T	208/189	34.1/6.5	11	0.955	0.98 (0.81-1.18)	0.809	17.4/4.7	8	0.916	0.97 (0.80-1.17)	0.711
	rs3762993	C/C	93/85	34.4/5.2	11		1.00		12.1/3.5	8		1.00	
		C/T	278/251	30.9/8.3	11		1.13 (0.88-1.46)	0.337	16.9/8.4	8		1.19 (0.92-1.54)	0.183
		T/T	222/192	33.1/10.5	11	0.519	1.08 (0.89-1.30)	0.431	18.7/10.2	8	0.262	1.11 (0.92-1.34)	0.265
	rs9324677	C/C	196/178	30.6/7.1	11		1.00		16.3/6.7	8		1.00	
		A/C	289/251	32.4/10.1	11		1.02 (0.80-1.31)	0.882	19.6/7.9	8		0.97 (0.76-1.24)	0.810
		A/A	104/96	34.6/6.6	12	0.893	0.98 (0.77-1.24)	0.844	13.7/6.5	8	0.908	0.95 (0.75-1.21)	0.696
	rs13185706	A/A	481/427	32.2/9.1	11		1.00		19.0/9.6	8		1.00	
		C/A	104/93	33.2/5.9	11		0.83 (0.41-1.67)	0.594	12.0/6.1	8		0.82 (0.41-1.65)	0.579
		C/C	8/8	25.0/-	11	0.761	0.87 (0.42-1.79)	0.706	/-	8	0.734	0.87 (0.42-1.78)	0.694
	rs888988	G/G	415/369	32.4/9.1	11		1.00		17.1/-	8		1.00	
		C/G	159/145	28.9/-	10		1.79 (1.05-3.05)	0.033	13.8/-	8		1.80 (1.05-3.07)	0.031
		C/C	19/14	36.8%/-	15	0.026	1.97 (1.14-3.41)	0.016	36.8/21.1	10	0.018	1.99 (1.15-3.45)	0.014
I-II	rs11960458	C/C	109/101	26.6/0.5	11		1.00		20.3/12.5	8		1.00	
		T/C	195/170	34.1/10.5	12		1.44 (1.04-1.98)	0.027	13.0/6.6	8		1.37 (1.00-1.90)	0.053
		T/T	74/59	39.2/-	12	0.052	1.22 (0.91-1.64)	0.187	46.2/15.4	8	0.097	1.20 (0.89-1.62)	0.226
Age>40	rs888988	G/G	230/217	27.4/3.7	11		1.00		14.5/4.2	8		1.00	
		C/G	89/79	30.3/10.0	10		2.28 (1.12-4.63)	0.022	15.7/-	8		2.30 (1.13-4.66)	0.021
		C/C	12-8	58.3/33.3	13	0.034	2.22 (1.07-4.61)	0.032	41.7/33.3	9	0.026	2.20 (1.06-4.55)	0.034
Male	rs888988	G/G	241/216	33.2/7.6	11		1.00		19.0/8.4	8		1.00	
		C/G	72/65	30.6/6.7	10		2.67 (1.18-6.02)	0.018	16.7/7.4	7		2.61 (1.16-5.9)	0.021
		C/C	11-6	63.6/43.6	19	0.016	2.93 (1.27-	0.012	63.3/43.5	14	0.013	2.94	0.012

OS: overall survival; PFS: progression free survival; ST: survival rate; MST: median survival time

HR: hazard ratio; 95% CI: 95% confidence interval

$p < 0.05$ indicates statistical significance.

Figures

Figure 1

Haplotype block map for the SNPs in the ANXA6 gene Standard color frame is used to show LD pattern. Red squares display statistically significant associations between a pair of SNPs, as measured by D'; darker shades of red indicate higher D'.

Figure 2
Kaplan–Meier curves for overall survival and progression-free survival according to age, extent of resection and chemotherapy in patients with glioma

Figure 3
Kaplan–Meier curves for overall survival and progression-free survival base on ANXA6 polymorphisms in patients with glioma