FXYD2 mRNA Expression Represents a New Independent Factor That Affects Survival of Glioma Patients and Predicts Chemosensitivity of Patients to Temozolomide

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Abstract

Glioma is the most common primary intracranial tumor. Owing to the poor prognosis associated with high-grade gliomas, there is an urgent need to identify biomarkers related to prognosis and treatment sensitivity. Clinical features, FXYD2 mRNA expression levels, and survival data were analyzed for 1265 glioma samples from the Chinese Glioma Genome Map Project and two independent databases. The expression patterns for FXYD2 mRNA were compared using the chi-square test, and overall survival (OS) of glioma patients was evaluated according to FXYD2 mRNA expression levels. The factors affecting glioma survival were evaluated by Cox univariate and multivariate regression analysis. We found patients with primary oligodendroglioma, low World Health Organization (WHO) grade, low WHO molecular grade, isocitrate dehydrogenase (*IDH*) mutation, and combined deletion of 1p19q showed higher FXYD2 mRNA expression and longer survival times. Moreover, temozolomide (TMZ) chemotherapy was found to be an independent factor affecting survival in patients with high FXYD2 mRNA expression, but not in patients with low expression. So FXYD2 mRNA expression represents a new independent factor affecting the survival of glioma patients and may serve as an independent prognostic indicator to predict the sensitivity of gliomas to TMZ.

Introduction

Glioma, the most common primary malignant tumor of the brain [1], was classified in 2016 by the World Health Organization (WHO) based on co-deletion of the molecular markers, isocitrate dehydrogenase (*IDH*) and 1p19q [2]. Moreover, the prognosis of high-grade gliomas remains poor, particularly in patients with glioblastoma who have a 5-year survival rate of only 5% [1,3], even after administration of the standard three treatments: maximum surgical resection, radiotherapy, and chemotherapy. In fact, the average survival time for glioblastoma patients is only 14 months [4], which is caused not only by the difficulty associated with treating the heterogenous tumors [5] but also by the increasing level of resistance reported for temozolomide (TMZ), which renders this standard glioblastoma chemotherapeutic drug ineffective[6–9]. Meanwhile, the application of high-throughput technology for the molecular classification of gliomas as well as for screening differentially expressed genes and drug resistance genes has become a research hotspot to facilitate the development of corresponding targeted drugs.

Na/K-ATPase is an oligomeric transmembrane protein composed of α, β, and γ subunits that functions to maintain the dynamic membrane potential and is associated with many cellular functions as well as the pathogenesis of specific diseases [10]. Specifically, Na/K-ATPase upregulation has been reported in various cancers [11–15]. Meanwhile, inhibiting Na/K-ATPase activation and expression effectively inhibits cancer cell proliferation and survival [16–17]. FXYD2 (sodium/potassium-transporting ATPase subunit gamma) is the γ subunit of the Na/K-ATP enzyme and functions as a regulator of the enzyme activity [18]. Interestingly, a previous study reported that in ovarian clear cell carcinoma (CCC) patients, the expression level of FXYD2 was positively correlated with patient prognosis. Specifically, upregulated FXYD2 expression increased the sensitivity of ovarian CCC cells to the Na/K-ATPase inhibitor cardiotonic

glycoside, thereby enhancing its therapeutic effect. However, the expression pattern and clinical significance of FXYD2 have not yet been reported in gliomas.

Here, through transcriptome sequencing, this study sought to establish the relationship between FXYD2 mRNA expression and the clinical features and survival data for glioma cases collected from the Chinese Glioma Genome Map Project as well as TCGA and REMBRANDT databases.

Results

Clinical Features and FXYD2 mRNA Expression in 516 Glioma Patients in CGGA.

Clinical features included sex, age, recurrence status, histopathology, WHO grade, IDH mutation status, 1p/19q co-deletion status, radiotherapy status, chemotherapy, and WHO classification (2016). The patients were divided into two groups according to a median age of 43 years. The 516 glioma patients were then classified as having low or high FXYD2 mRNA expression based on median expression values (Table 1). The median expression value was ≥ the median expression value and < median expression value (Table 1).

Table 1
Relationship between FXYD2 mRNA expression and clinical features in 516 glioma patients

Parameter	Variable	N	FXYD2 mRNA expression				χ2	P value
			Low	%	High	%		
Sex	Female	225	115	51.1	110	48.9	0.197	0.657
	Male	291	143	49.1	148	50.9		
Age*	< 43	238	119	50.0	119	50.0	0.000	1.000
	≥ 43	278	139	50.0	139	50.0		
Progression status	Primary	301	129	42.9	172	57.1	14.743	0.000
	Recurrent	215	129	60.0	86	40.0		
Histopathological types	oligodendroglioma	114	31	27.2	83	72.8	31.937	0.000
	astrocytoma	218	117	53.7	101	46.3		
	glioblastoma	184	110	59.8	74	40.2		
WHO classification	II	134	60	44.8	74	55.2	10.951	0.004
	III	198	88	44.4	110	55.6		
	IV	184	110	59.8	74	40.2		
IDH mutation status	Wildtype	224	155	69.2	69	30.8	58.347	0.000
	Mutant	292	103	35.3	189	64.7		
1p/19q codeletion status	Non-codel	401	231	57.6	170	42.4	41.636	0.000
	Codel	115	27	23.5	88	76.5		
Radiotherapy	No	108	54	50.0	54	50.0	0.000	1.000
	Yes	408	204	50.0	204	50.0		
Chemotherapy	No	124	60	48.4	64	51.6	0.170	0.680
	Yes	392	198	50.5	194	49.5		
WHO classification (2016)	IDH Mutant, 1p/19q Codel (LGG)	101	22	21.8	79	78.2	72.323	0.000

*The patients were divided into two groups according to the median age of 43 years.

Parameter	Variable	N	FXYD2 mRNA expression				χ2	P value
			Low	%	High	%		
	IDH Mutant, 1p/19q Non-codel (LGG)	154	68	44.2	86	55.8		
	IDH Wildtype (LGG)	77	58	75.3	19	24.7		
	IDH Mutant (GBM)	37	13	35.1	24	64.9		
	IDH Wildtype (GBM)	147	97	66.0	50	34.0		
*The patients were divided into two groups according to the median age of 43 years.								

Relationship Between FXYD2 mRNA Expression and Clinical Features in Glioma Patients.

The relationship between clinical characteristics and FXYD2 mRNA expression was analyzed. FXYD2 mRNA expression was not associated with sex (P = 0.657), age (P = 1.000) or radiotherapy or chemotherapy status (P = 1.000, P = 0.680, respectively) in glioma patients. It was, however, significantly correlated with recurrence (P = 0.000), histopathology (P = 0.000), WHO grade (P = 0.004), *IDH* mutation (P = 0.000), *1p/19q* co-deletion (P = 0.000), and WHO molecular grade (2016; P = 0.000). Patients with good prognostic indicators, such as primary, oligodendroglioma, low WHO grade, *IDH* mutation, *1p/19q* co-deletion, and low WHO molecular grade had increased FXYD2 mRNA expression (Table 1).

FXYD2 MRNA Expression Is Higher in Glioma Patients With Better Prognosis.

FXYD2 mRNA expression in glioma patients with different clinical and molecular pathological features was compared using a scatter plot. The clinical features assessed included sex, age, recurrence (Fig. 1), histopathology, WHO grade (Fig. 2), *IDH* mutation, *1p/19q* co-deletion status, and 2016 WHO molecular grade (Fig. 3). The patients were divided into two groups according to the median age of 43 years. The results showed that the expression of FXYD2 mRNA was higher in patients with a good prognosis, including those with primary glioma (P = 0.00031), oligodendroglioma (P = 5.6e-10), WHO low grade (P = 0.00011), *IDH* mutation (P = 2.5e-18), *1p/19q* co-deletion (P = 5.3e-12), and low WHO molecular grade (2016) (P = 2.3e-20).

Moreover, the survival time for glioma patients with high FXYD2 mRNA expression was longer. Kaplan–Meier survival curves were used to explore the effect of FXYD2 mRNA expression on the total survival time of glioma patients. The results show that the survival time of patients with high expression of FXYD2 mRNA was longer than that of patients with low expression of WHOII (P = 0.000; Fig. 4A). After stratifying the data according to WHO grade, the same result was observed in patients with all grades of glioma: WHOII (P = 0.011; Fig. 4B), WHOIII (P = 0.000; Fig. 4C), and WHOIV (P = 0.043; Fig. 4D). The same results were also obtained for patients with primary initial gliomas (P = 0.000; Fig. 4E) and relapse (P = 0.000; Fig. 4F).

FXYD2 mRNA Expression Can Predict the Survival and Prognosis of Glioma Patients.

Subgroup analysis showed that different subgroups of glioma patients with high FXYD2 mRNA expression also had longer OS. Among them, low-grade glioma (P = 0.011), high-grade glioma (P = 0.000), oligodendroglioma (P = 0.004), astrocytoma (P = 0.000), *IDH* mutant type (P = 0.000), *IDH* wild type (P = 0.180), *1p/19q* co-deletion type (P = 0.033), and *1p/19q* non-co-deletion type (P = 0.000) (Fig. 5).

FXYD2 mRNA Expression can Predict the Survival and Prognosis of Glioma Patients in Two Independent Databases.

Using Kaplan–Meier survival curves, it was confirmed in two independent databases that glioma patients with high FXYD2 mRNA expression had a longer survival time than patients with low expression from TCGA (P = 0.000, Fig. 6A) and REMBRANDT database (P = 0.000, Fig. 6B). Further subgroup analysis on the two independent datasets showed that patients with low- or high-grade gliomas that had high FXYD2 mRNA expression also exhibited longer survival times (Fig. 6C–F).

FXYD2 mRNA Expression is an Independent Factor Affecting the Survival of Glioma Patients.

Univariate Cox analysis was used to identify the factors affecting the survival of glioma patients, including sex, age, recurrence, histopathology, WHO grade, *IDH* mutation status, *1p/19q* co-deletion status, radiotherapy or chemotherapy status, and FXYD2 mRNA expression. Multivariate Cox analysis showed that high FXYD2 mRNA expression (HR: 0.751, 95 %CI 0.583–0.967, P = 0.026), *IDH* mutation (HR: 0.714, 95% CI: 0.532–0.959, P = 0.025), *1p/19q* co-deletion (HR: 0.379, 95% CI: 0.253–0.567, P = 0.000), and chemotherapy status (HR: 0.615, 95% CI: 0.452–0.835, P = 0.002) were independent factors associated with longer patient survival. Meanwhile, age (HR: 1.015, 95% CI: 1.005–1.024, P = 0.002), relapse (HR: 2.081, 95% CI: 1.650–2.625, P = 0.000), and WHO grade (HR: 2.754, 95% CI: 1.972–3.847, P = 0.000) represented independent factors associated with poor survival (Table 2).

Table 2
Correlation analysis between FXYD2 mRNA expression and overall survival among 516 glioma patients in CGGA. HR = hazard ratio; CI = confidence interval.

Parameter	Univariate Cox Regression			Multivariate Cox Regression		
	P value	HR	95% CI (low-up)	P value	HR	95% CI (low-up)
Sex	0.447	1.091	0.871–1.366			
Age	0.000	1.027	1.017–1.036	0.002	1.015	1.005–1.024
Recurrent	0.000	2.117	1.690–2.651	0.000	2.081	1.650–2.625
Histopathological types	0.000	1.827	1.657–2.015	0.225	0.876	0.708–1.085
WHO classification	0.000	2.804	2.377–3.308	0.000	2.754	1.972–3.847
IDH mutation status	0.000	0.321	0.256–0.403	0.025	0.714	0.532–0.959
1p/19q codeletion status	0.000	0.282	0.199–0.400	0.000	0.379	0.253–0.567
Radiotherapy	0.467	1.109	0.839–1.465	0.221	0.824	0.604–1.123
Chemotherapy	0.230	1.176	0.903–1.533	0.002	0.615	0.452–0.835
FXYD2 mRNA expression	0.000	0.469	0.374–0.589	0.026	0.751	0.583–0.967

Increased FXYD2 mRNA Expression Can Predict the Chemosensitivity of Glioma Patients.

According to the median expression of FXYD2 mRNA, the patients were divided into two groups: low or high expression. Univariate Cox analysis was used to investigate the related factors affecting the survival time of glioma patients, including sex, age, recurrence, histopathology, WHO grade, *IDH* mutation status, *1p/19q* co-deletion status, radiotherapy, and chemotherapy status. The results of multivariate Cox analysis showed that in the group with high FXYD2 mRNA expression, chemotherapy status (HR: 0.427, 95% CI: 0.262–0.695, P = 0.001), *IDH* mutation status (HR: 0.498, 95% CI 0.316–0.783, P = 0.003), and *1p/19q* co-deletion (HR: 0.405, 95% confidence interval 0.229–0.716, P = 0.002) were independent factors associated with longer survival. Meanwhile, age (HR value: 1.016, 95% CI: 1.000–1.031, P = 0.043), relapse (HR value: 2.669, 95% CI: 1.832–3.889, P = 0.000), WHO grade (HR value: 3.749, 95% CI: 2.109–6.663, P = 0.000) were independent factors associated with poor survival (Table 3). However, in the group with low FXYD2 mRNA expression, chemotherapy status was not an independent factor affecting the survival of patients with glioma (univariate Cox analysis P = 0.132, multivariate Cox analysis P = 0.192; Table 3).

Table 3
Correlation analysis between chemotherapy and overall survival among glioma patients with high and low FXYD2 mRNA expression in CGGA. HR = hazard ratio; CI = confidence interval.

Parameter	Univariate Cox Regression			Multivariate Cox Regression		
	P value	HR	95% CI (low-up)	P value	HR	95% CI (low-up)
High						
Sex	0.002	1.792	1.231–2.607	0.096	1.418	0.940–2.138
Age	0.000	1.031	1.015–1.047	0.043	1.016	1.000–1.031
Recurrent	0.000	2.041	1.432–2.910	0.000	2.669	1.832–3.889
Histopathological types	0.000	2.037	1.754–2.365	0.472	0.879	0.618–1.250
WHO classification	0.000	3.589	2.727–4.723	0.000	3.749	2.109–6.663
IDH mutation status	0.000	0.261	0.181–0.375	0.003	0.498	0.316–0.783
1p/19q codeletion status	0.000	0.309	0.199–0.480	0.002	0.405	0.229–0.716
Radiotherapy	0.256	1.300	0.827–2.043	0.359	0.781	0.461–1.324
Chemotherapy	0.909	1.024	0.682–1.537	0.001	0.427	0.262–0.695
Low						
Sex	0.101	0.786	0.589–1.048			
Age	0.000	1.023	1.011–1.035	0.028	1.013	1.001–1.025
Recurrent	0.000	1.880	1.399–2.525	0.000	1.856	1.374–2.508
Histopathological types	0.000	1.583	1.387–1.807	0.236	0.849	0.648–1.113
WHO classification	0.000	2.222	1.818–2.716	0.000	2.472	1.630–3.750
IDH mutation status	0.000	0.472	0.347–0.642	0.398	0.851	0.586–1.237
1p/19q codeletion status	0.001	0.363	0.197–0.668	0.009	0.426	0.225–0.808
Radiotherapy	0.861	0.969	0.679–1.382	0.253	0.797	0.541–1.176
Chemotherapy	0.132	1.310	0.922–1.862	0.192	0.764	0.510–1.145

Discussion

The *FXYD2* gene is located on chromosome 11q23 [21], while the FXYD2 protein is the r subunit of the Na-K-ATP enzyme. FXYD2 has been shown to reduce the Na ion affinity of Na-K-ATP [22], resulting in

subsequent inhibition of cell proliferation [23]. However, the expression and application value of FXYD2 mRNA in gliomas have not been previously reported.

This study revealed that the expression of FXYD2 mRNA is related to the degree of malignancy of gliomas. Specifically, higher degree malignancies are associated with lower FXYD2 mRNA expression, suggesting that FXYD2 mRNA expression can be used as a predictive biomarker for the degree of malignancy of gliomas. Moreover, FXYD2 mRNA expression was found to be related to the survival time of glioma patients with lower expression associated with shorter survival time, suggesting that it can also be used to predict patient survival prognosis. FXYD2 mRNA expression was also related to the chemosensitivity of glioma patients to TMZ. Meanwhile, TMZ represents an independent factor affecting the survival of glioma patients with high expression of FXYD2 mRNA, but not patients with low expression. Hence, we postulate that the expression of FXYD2 mRNA can be used to predict the chemosensitivity to TMZ. Specifically, patients with high FXYD2 mRNA expression will be more likely to respond to TMZ therapy, thereby prolonging survival time, while those with low expression will not benefit from this therapy. These results were similar to those reported by Hsu I-Ling et al [24] who found that, compared with ovarian cancer cells expressing low levels of FXYD2, those with high expression were more sensitive to cardiosides, while cardiotonic glycosides can effectively inhibit the growth of ovarian cancer cells.

Currently, the underlying mechanism associated with the effects of FXYD2 in tumors is unclear. However, the Na-K-ATPase serves as the transport system for Na and K ions on the cell membrane [25], which serves to maintain the Na/K ion concentration gradient inside and outside of the cell. These gradients are essential for maintaining cell volume and membrane potential and also guarantee the maintenance of intracellular homeostasis[25]. They also provide nutrients to the cells and regulate the concentration of intracellular pH and calcium ions. Meanwhile, increased expression of FXYD2 was found to decrease the activity of Na/K-ATPase, causing us to speculate that the increase in FXYD2 decreased Na/K-ATPase activity, thereby disrupting the ion homeostasis inside and outside of the tumor cell membrane while also decreasing the nutritional supply to tumor cells and subsequently inhibiting tumor cell proliferation causing tumor cells to become more unstable and vulnerable to chemotherapeutic drugs. However, these hypotheses must be verified by further investigations.

Moreover, another study has reported that the body senses mechanical pain abnormalities caused by peripheral inflammation through FXYD2 in neurons [26]. Following peripheral tissue inflammation, the interaction between FXYD2 and the α subunit of Na/K-ATPase is enhanced, causing downregulation of Na/K-ATPase activity, while increasing neuronal membrane potential depolarization and excitability. The body then senses peripheral inflammatory stimulation signals, resulting in corresponding inflammatory stress and clearance responses. We, therefore, speculate that FXYD2 also exerts anti-tumor effects by enhancing the inflammatory response of the body toward tumors. However, this hypothesis also requires further verification.

Methods

Data Collection. Data, including clinical information (sex, age), histopathology, WHO grade, molecular pathology (*IDH* mutation, *1p/19q* deletion), and follow-up information (survival time), of 516 glioma patients were collected from the Chinese glioma Genome Map Project (CGGA). All patients were classified according to the World Health Organization (WHO) molecular classification in 2016 [2]. The overall survival (OS) rate of clinical end-point events was calculated from the initial pathological diagnosis to death or last follow-up. This study was approved by the Institutional Ethics Committee of Beijing Tiantan Hospital (KYSB 2015–017), and all patients provided written informed consent.

mRNA Sequencing.

mRNA Transcriptome Sequencing. According to the manufacturer's instructions, total RNA was extracted with an RNeasy Mini Kit (Qiagen). Pestle and QIAshredder (Qiagen) were used to crush and homogenate the frozen tissue. The RNA integrity was assessed via electrophoresis using the 2100 bioanalyzer (Agilent Technologies), and only high-quality samples with RNA integrity numbers (RIN) ≥ 6.8 were used to construct the sequencing library. Briefly, 1 μg of total RNA was used in conjunction with the TruSeq RNA library preparation kit (Illumina). With the exception of SuperScript III reverse transcriptase (Invitrogen) used the synthesis of the first strand of cDNA, all other operations were low-throughput. Following PCR amplification, and purification of the junction fragments, the DNA concentration of the junction was determined by quantitative PCR (biological system 7500) with QP1 5′-AATGATACGGCGACCACCGA-3′ primers and QP2 5′-CAAGCAGAAGACGGCATACGAGA-3′ primers. The length of the DNA fragment was measured using a 2100 bioanalyzer, and the median size of the inserted fragment was 200 bp. The RNA-seq library was sequenced using the Illumina HiSeq 2000 Universe 2500 Universe 4000 sequencing system. The library adopts a paired end strategy, with reading lengths of 101 bp, 125 bp, or 150 bp. Base invocation was performed using the Illumina Casava v1.8.2 pipeline.

Mapping and Quantification. STAR (v2.5.2b, Dobin et al., 2012) and RSEM (v1.2.31, Li et al., 2011) software were used for RNA-seq mapping and quantification. These reads were then compared with the Human Genome reference (GENCODE v19, hg19) for STAR, after which RSEM was used to calculate the sequencing reads for each GENCODE gene. The expression levels of different samples were combined into an FPKM matrix (fragments per million fragments per kilobase transcriptome). Only when the expression level was > 0 in half the samples was a gene defined as expressed. Finally, we retained only the expressed genes in the mRNA expression profile.

RNA-Seq Comparison Workflow. STAR (v2.5.2b) was used to compare the mRNA profiles. For each RNA-seq sample, STAR compares each read group with the human reference genome (GENCODE v19, hg19), and then merges the alignment results. This workflow generates a BAM file that contains both aligned and unaligned reads (against data). All experimental methods were carried out in accordance with the relevant guidelines and regulations that were previously reported [19]. Preparation, sequencing, and data analysis of the RNA-seq library were the same as that described previously[20].

Verification Group Data Collection. The clinical, histopathological, and survival follow-up data, as well as FXYD2 mRNA sequencing data, for glioma patients were collected from two open independent datasets. Among them, 481 cases were from the cancer genome map database (TCGA, http://cancergenome.nih.gov), and 268 were from the molecular brain tumor database (REMBRANDT, http://caintegrator-info.nci.nih.gov).

Statistical Analysis. R software 3.3.2 and SPSS software 25.0 were used to perform all statistical analyses and to generate box scatter plots and survival curves. The normally distributed data were expressed as mean ± standard deviation (x ± s). Student's t-tests, one-way ANOVA, and LSD-t pairwise comparisons were used to compare FXYD2 mRNA expression in different groups. Kaplan–Meier curve and log rank test were used to analyze the OS of patients in different groups. Univariate and multivariate Cox regression analyses were used to analyze the factors affecting the survival time of glioma patients. All statistical analyses were bilateral, and results were considered statistically significant at $P < 0.05$.

Conclusions

This study revealed that the expression of FXYD2 mRNA in gliomas can predict the degree of malignancy and survival time of patients. At the same time, FXYD2 mRNA expression can predict the chemosensitivity of glioma patients to TMZ. However, considering that our study is limited to mRNA, the transcriptional regulation, protein translation, as well as underlying regulatory mechanisms and pathways of FXYD2 remain unclear and require further investigation.

Declarations

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Author contributions statement

Fang Wang performed the experiments and analysis, collected and interpreted the data, and wrote the manuscript. Kaijia Zhou, Tao Jiang, Hui Liang, and Ming Zhang collected the data. Kaijia Zhou designed the study and edited the manuscript. All authors reviewed the manuscript.

Competing Interests

The authors declare that manuscript, or any part of it, has not been previously published or submitted concurrently to any other journal.

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Figures

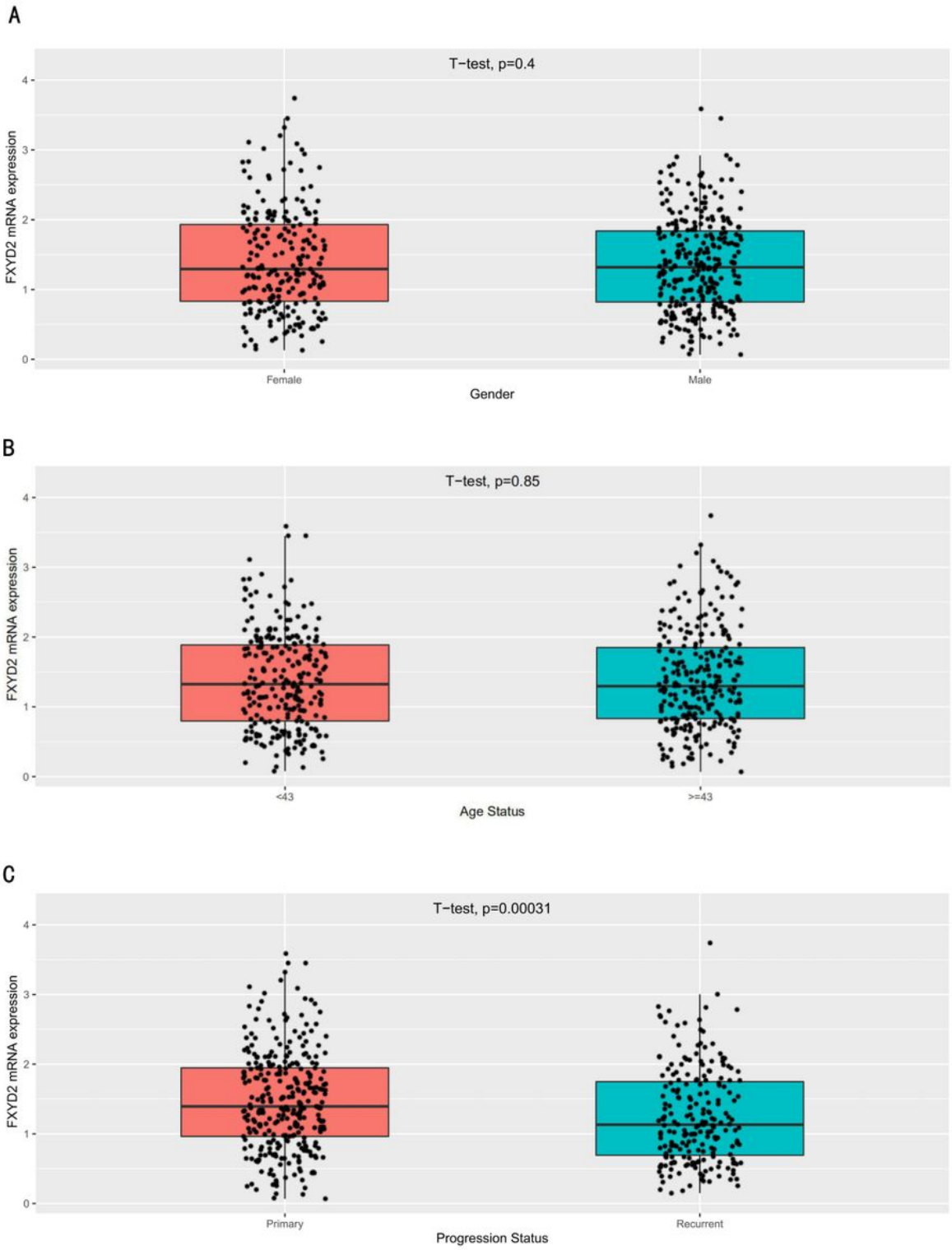

Figure 1

Analysis of FXYD2 mRNA expression according to sex, age, and progression status in CGGA. Comparison of sex (A), age (B), and progression status (C). n = 516 per group. Data represent mean ± SD. Significance determined by Student's t-test.

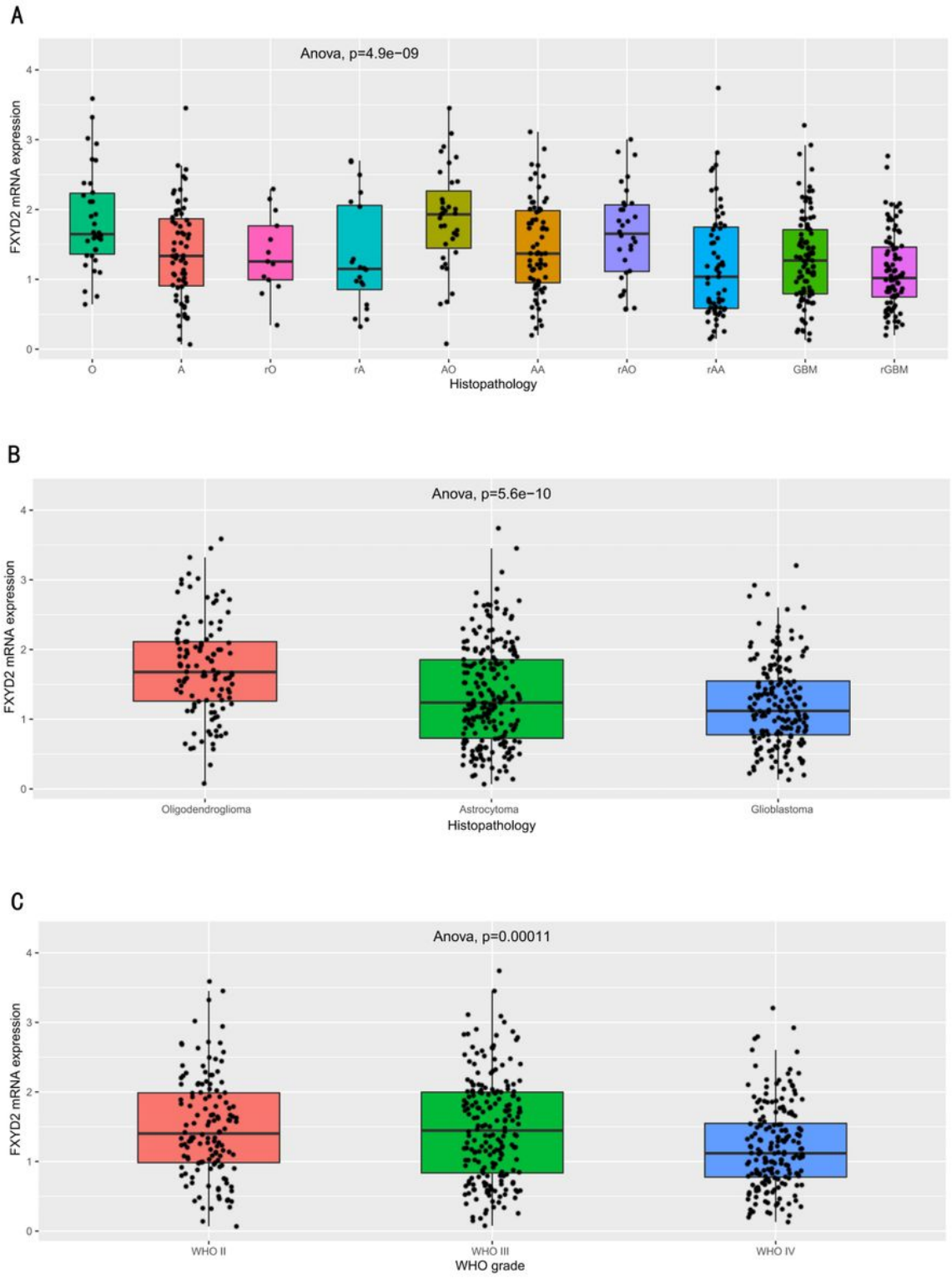

Figure 2

Analysis of FXYD2 mRNA expression according to histopathology and WHO grade in CGGA. Comparison of histopathology (A, B) and WHO grade (C). n = 516 per group. Data represent mean ± SD. Significance determined by ANOVA.

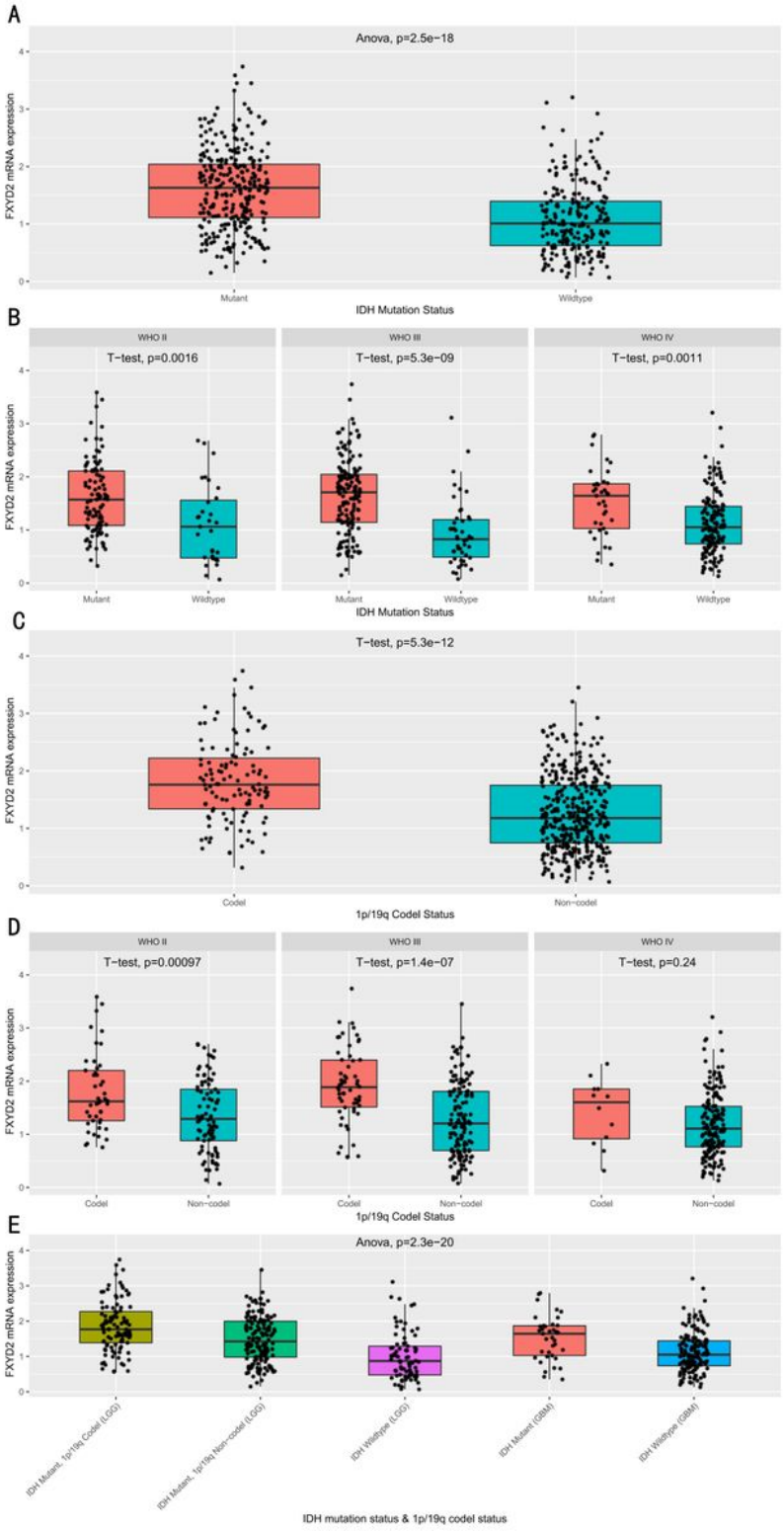

Figure 3

Analysis of FXYD2 mRNA expression according to IDH mutation and 1p/19q col-deletion status. Subgroup analysis of FXYD2 mRNA expression according to WHO classification in CGGA. Different IDH mutation statuses (A) and 1p/19q deletion statuses (C) were compared. Subgroup analysis of FXYD2 mRNA expression (B, D). Comparison of WHO molecular classification of glioma in 2016 (E). In (A, C, E), n

= 516. In (B, D), n (II) = 134, n (III) = 198, n (IV) = 184. Data represent mean ± SD. In (A-D) significance was determined by Student's t-test. In (E), significance was determined by ANOVA.

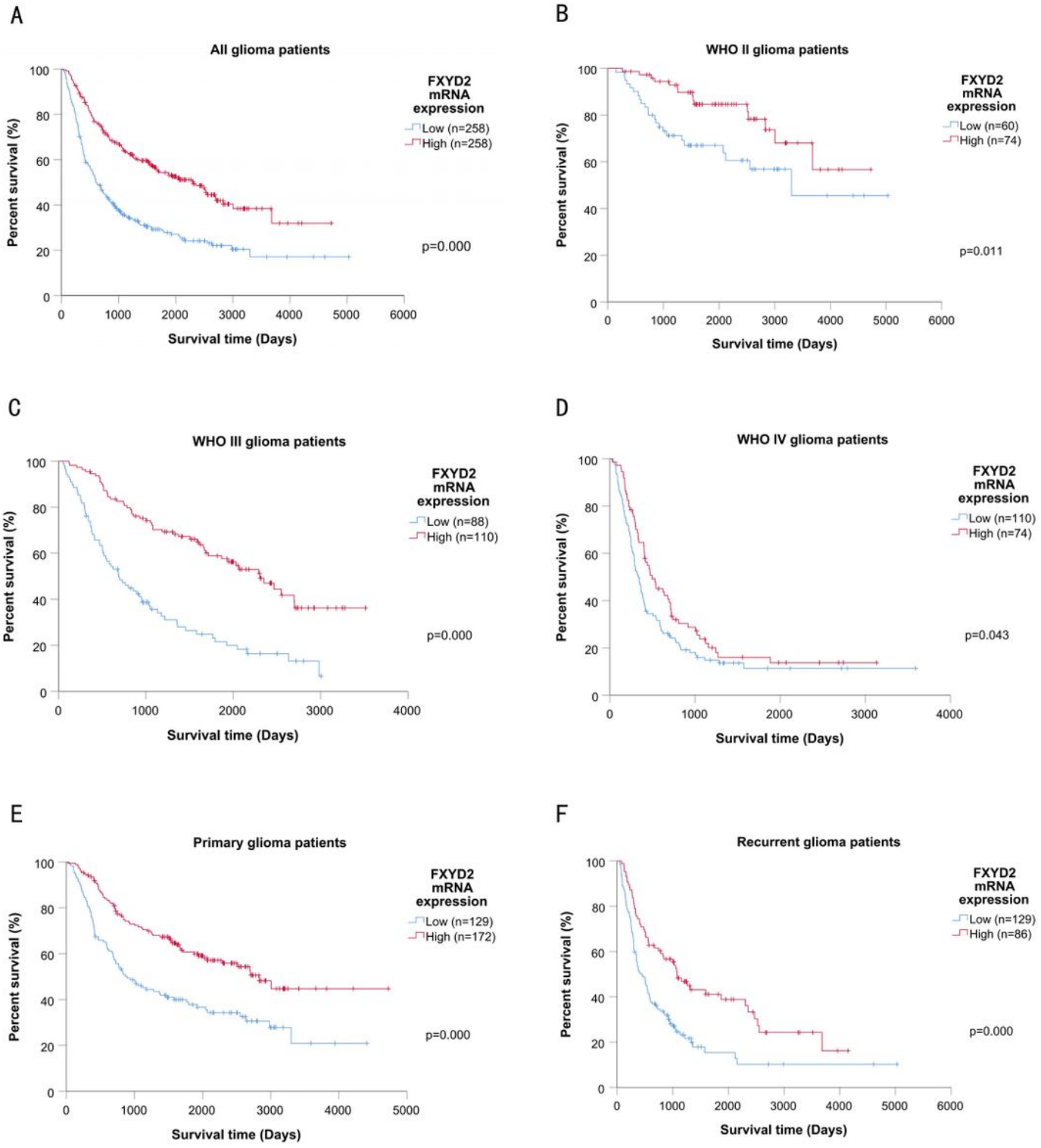

Figure 4

Kaplan–Meier curves for overall survival (OS) according to FXYD2 mRNA expression in CGGA. Subgroup analysis of OS performed based on Kaplan–Meier curves according to WHO classification and

progression status. Kaplan–Meier curves for OS according to WHO classification (A–D) and progression status (E, F). Significance determined by Log Rank (Mantel-Cox) test.

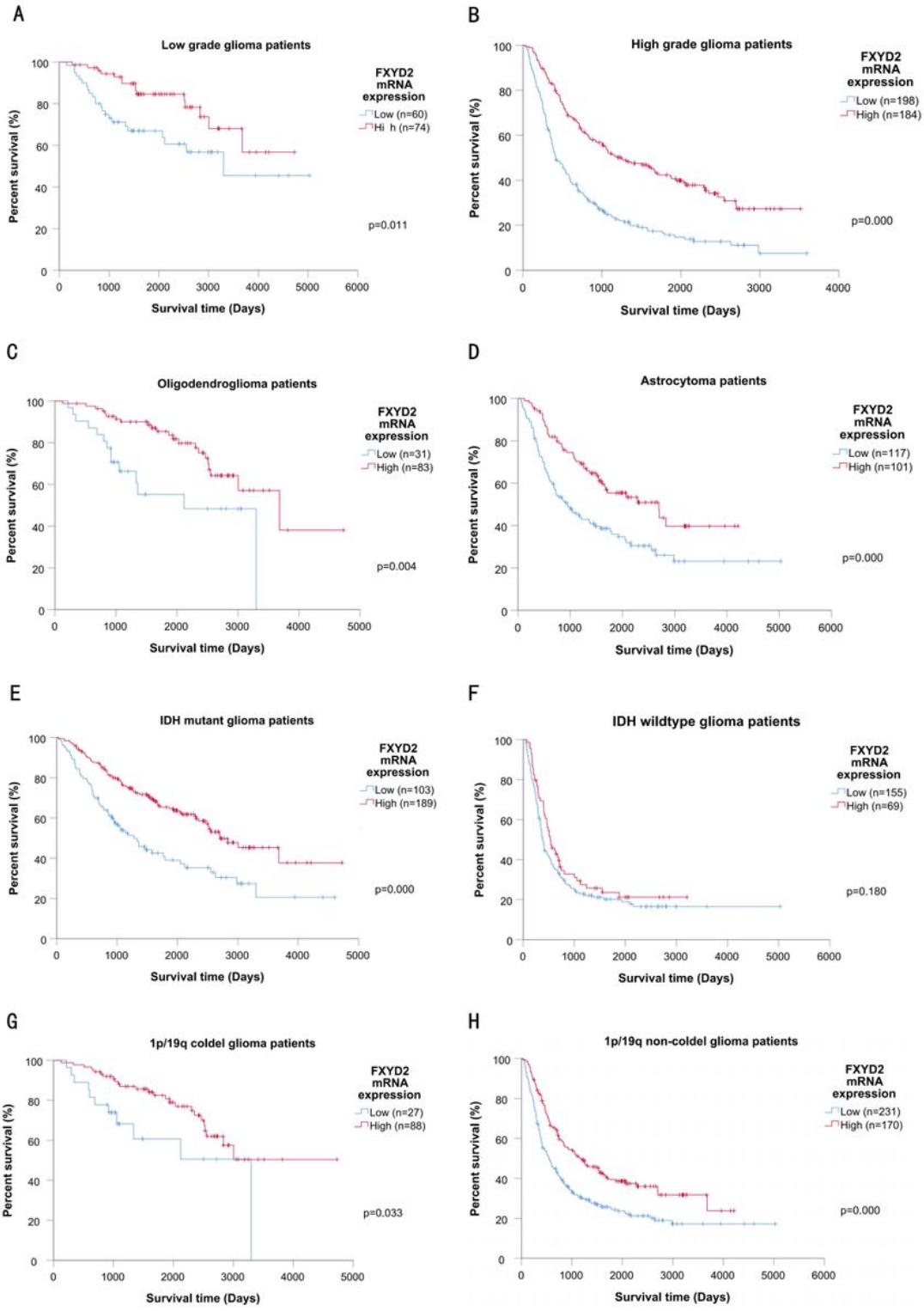

Figure 5

Kaplan–Meier curves for overall survival (OS) according to FXYD2 mRNA expression in CGGA. Subgroup analysis of OS performed based on Kaplan–Meier curves according to histologic type, WHO classification, IDH mutation and 1p/19q col-deletion status. Kaplan–Meier curves for OS according to

WHO classification (A, B), histologic type (C, D), IDH mutation (E, F) and 1p/19q col-deletion status (G, H). Significance determined by Log Rank (Mantel-Cox) test.

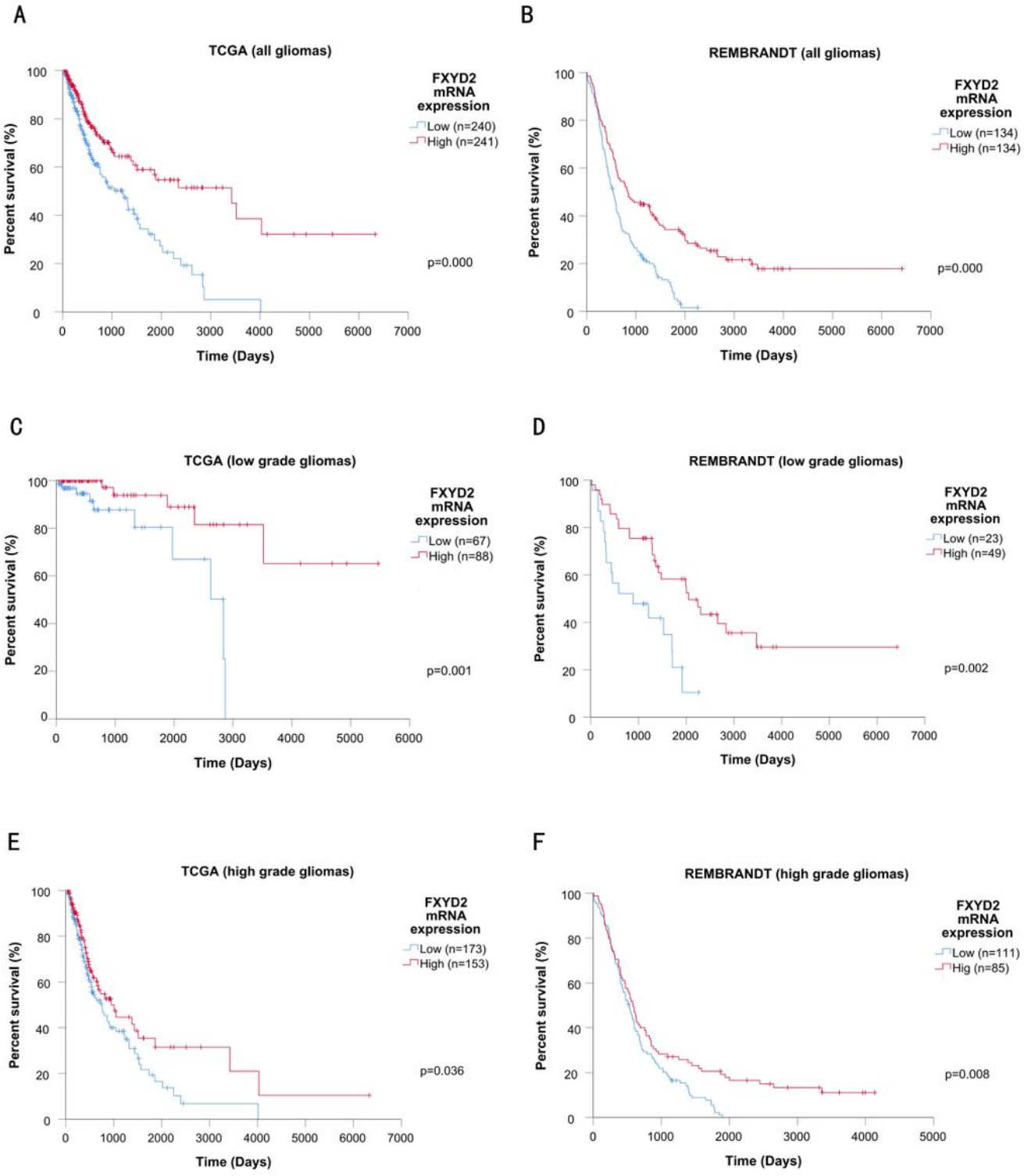

Figure 6

Kaplan–Meier curves for overall survival (OS) according to FXYD2 mRNA expression in TCGA and REMBRANDT databases. Subgroup analysis of OS performed based on Kaplan–Meier curves according

to WHO classification. Kaplan–Meier curves for OS in TCGA (A, C, E) and REMBRANDT (B, D, F) databases. Significance determined by Log Rank (Mantel-Cox) test.