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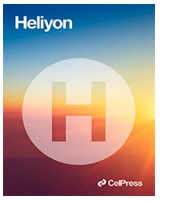

Research article

Clinical sequencing reveals diagnostic, therapeutic, and prognostic biomarkers for adult-type diffuse gliomas

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ABSTRACT

Diffuse gliomas in adults are highly infiltrative and largely incurable. Whole exome sequencing (WES) has been demonstrated very useful in genetic analysis. Here WES was performed to characterize genomic landscape of adult-type diffuse gliomas to discover the diagnostic, therapeutic and prognostic biomarkers. Somatic and germline variants of 66 patients with adult-type diffuse gliomas were detected by WES based on the next-generation sequencing. TCGA and CGGA datasets were included to analyze the integrated diagnosis and prognosis. Among 66 patients, the diagnosis of 9 cases was changed, in which 8 cases of astrocytoma were corrected into *IDH*-wildtype glioblastoma (GBM), and 1 oligodendroglioma without 1p/19q co-deletion into astrocytoma. The distribution of mutations including *ATRX/TP53* differed in three cohorts. The genetic mutations in GBM mainly concentrated on the cell cycle, PI3K and RTK pathways. The mutational landscape of astrocytoma was more similar to that of GBM, with the highest frequency in germline variants. Patients with *IDH*-mutant astrocytoma harboring SNVs of *PIK3CA* and *PIK3R1* showed a significantly worse overall survival (OS) than wild-type patients. *AEBP1* amplification was associated with shorter OS in GBM. Our study suggests that clinical sequencing can recapitulate previous findings, which may provide a powerful approach to discover diagnostic, therapeutic and prognostic markers for precision medicine in adult-type diffuse gliomas.

1. Background

Gliomas are the most common brain malignancies, approximately accounting for 30 % of all primary brain tumors [1]. Due to the anatomic location in the brain, significant intratumoral heterogeneity, and fast developed therapy resistance, gliomas represent one of the most challenging malignancies [2]. The median survival time of glioma patients is 14–20 months even with standard of care therapeutics, which involves surgery, radiation, and/or chemotherapy. Gliomas are traditionally divided into histological grades I-IV. Recent advances in diagnostics and understanding of glioma molecular background have revolutionized its classification. Low grade glioma has been referred to grades I and II tumors while high grade glioma to grades III and IV. With advances in diagnostics and clinical experience, grade III IDH mutant gliomas are now considered lower grade gliomas in light of the patients' long survival time

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Abbreviations

GBM	glioblastoma
CNS	center nervous system
WHO	World Health Organization
WT	wildtype
OS	overall survival
WES	whole exome sequencing
NGS	next-generation sequencing
FFPE	formalin-fixed paraffin-embedded
TCGA	The Cancer Genome Atlas
CGGA	The Chinese Glioma Genome Atlas
BWA	Burrows-Wheeler Alignment
SNVs	single nucleotide variants
InDels	insertions and deletions
CNVs	copy number variants
OR	odds ratio
CI	confidence interval
HR	hazard ratio
FA	fanconi anaemia
HR	homologous recombination
NEJM	non-homologous end joining
BER	base excision repair
DR	direct repair
MMR	mismatch repair
NER	nucleotide excision
TLS	translesion synthesis
FISH	fluorescence *in situ* hybridization
TKIs	tyrosine kinase inhibitors; mAb: monoclonal antibodies

[3]. Adult-type diffuse gliomas are a group of gliomas with varying prognosis depending on the subtype and histology grade [4].

Molecular biomarkers have been proposed to define central nervous system (CNS) tumor entities in addition to histopathology since 2016 World Health Organization (WHO) classification of the CNS tumors [5]. Plentiful molecular parameters with clinico-pathologic utility that play important roles in accurate diagnosis are also incorporated in 2021 WHO classification of the CNS tumors (CNS5) [6]. The diagnostic alterations not only offer a reference for targeted treatment, but also are important for prognostic assessment [7]. According to *IDH1/2* and chromosome 1p/19q codeletion, adult-type diffuse gliomas are classified as *IDH*-mutant, 1p/19q codeleted oligodendroglioma; *IDH*-mutant, non-codeleted astrocytoma and *IDH*-wildtype (WT) GBM [8]. The molecular features of *IDH*-mutant, 1p/19q codeleted oligodendroglioma include *CIC*, *TERT*p, *FUBP1*, and *NOTCH1*, among which *CIC* and *FUBP1* mutations are uncorrelated with overall survival (OS) in oligodendroglioma, while *NOTCH1* mutations are significantly associated with worse OS [9]. *ATRX* and *TP53* inactivating mutations occur frequently in *IDH*-mutant astrocytoma, and homozygous deletion of cyclin-dependent kinase inhibitor (*CDKN2A/B*) is significantly associated with shorter OS [10]. The molecular characters of *IDH*-WT GBM are *TERT*p, gain of chromosome 7 and loss of chromosome 10 (+7/-10), and *EGFR* amplification [11]. In the WHO CNS5, the definition of tumor entities is emphasized using a few critical biomarkers except for morphology [12]. More alterations in key pathways still need to be identified to guide the patients appropriate for promising targeted therapies.

Whole exome sequencing (WES) based on next-generation sequencing (NGS) has been proven remarkably useful in genetic analysis appropriate for both screening previously investigated and novel significant variants [13]. The mutational landscape of gliomas is complex [14]. Therefore, it is clinically necessary to acquire comprehensive genomic data using WES for exploring diagnostic, prognostic and predictive biomarkers [15]. In the current study, we utilized WES to characterize genomic landscape of 66 patients with adult-type gliomas to discover the mutational biomarkers for precise diagnosis and potential treatment options for adult-type gliomas.

2. Materials and methods

2.1. Sample and clinical data collection

The clinical data of 66 patients with adult-type diffuse gliomas were collected at Xiangya Hospital from January 2018 to December 2021. Formalin-fixed paraffin-embedded (FFPE) tumor samples obtained by surgical resection were used for integrated histological and molecular diagnosis. Collection and analysis of the samples were approved by the Institutional Review Board of Xiangya Hospital Central South University, and were in accordance with local regulations and principles of Helsinki Declaration. Written informed consent forms were obtained from all patients for research, without restriction. Meanwhile, 824 and 144 patients with primary adult-

type diffuse gliomas who had determined *IDH* and 1p/19q status were collected from The Cancer Genome Atlas (TCGA) database and The Chinese Glioma Genome Atlas (CGGA), respectively.

2.2. WES

For each patient, genomic DNA from FFPE and paired blood control samples was extracted using Qiagen DNAeasy kits (Qiagen, USA). DNA was eluted and stored at −20 °C for further use. The quantification of DNA was done using Qubit 2.0 Fluorometer (Life Technologies, USA). Fragment analysis was done with the Agilent Genomic DNA ScreenTape assay kit on an Agilent 4200 TapeStation system (Agilent Technologies, USA). KAPA Hyper Prep Kit (KAPA Biosystems, USA) was used to prepare the sequencing libraries following the manufacturer's protocol. DNA was fragmented using enzymes, end prepped, ligated with adaptors and amplified. Hybridization was performed with Agilent Technologies SureSelect Human All Exon version 5 (Agilent Technologies, USA). Quantity and quality of libraries was evaluated with Qubit dsDNA HS assay (Thermo Fisher, Cat. Q32854). Subsequently, the library size distribution was assessed using the Agilent 4200 TapeStation system (Agilent Technologies, USA). Sequencing was performed on an Illumina NovaSeq 6000 apparatus (Illumina Inc., USA) with paired-end sequencing. The mean target coverage was set at 300x for tumor samples and 100x for paired normal samples.

2.3. Bioinformatics workflow

Data quality was evaluated with FastQC (v0.11.9). Adapters and low-quality reads were removed using fastp (v0.20). Sequencing reads were mapped to the human refence genome (hg19) using Burrows-Wheeler Alignment (BWA)-maximal exact matches algorithm, and default parameters were created to compress BAM files. The Genome Analysis Toolkit was used to realign the BAM files to improve the quality of alignment. Mutations including single nucleotide variants (SNVs), and insertions and deletions (InDels) were tested via Vardict [16], and their biological functions were annotated by ANNOVAR [17]. Synonymous mutations were not included in the mutation analysis. Known germline SNVs with a population frequency greater than 0.015 in databases such as dbSNP, 1000 Genomes, and ESP6500 were excluded from the analysis. Copy number variants (CNVs) were identified using CNVkit [18].

2.4. Statistical analysis

The differences of mutational frequencies and clinical factors were calculated by the two-sided Fisher's exact test, and the odds ratio (OR) with 95 % confidence interval (CI) was provided. Kaplan-Meier analysis based on log-rank test was executed to estimate survival curves, and the hazard ratio (HR) with 95 % CI was calculated using the Cox proportional hazards model. $P < 0.05$ was considered statistically significant for all hypothesis tests. All the statistical analyses and graphics were managed by computing software R v4.0.3.

Table 1
Baseline characteristics of the adult-type diffuse gliomas in three cohorts, n (%).

Characteristics	Xiangya cohort (n = 66)	TCGA (n = 824)	CGGA (n = 144)
Sex			
Male	42 (63.64)	448 (54.37)	83 (57.64)
Female	24 (36.36)	310 (37.62)	61 (42.36)
Unknown	0	66 (8.01)	0
Age, years	50 (25–69)	51 (18–89)	42 (19–76)
Histology			
Oligodendroglioma	9 (13.64)	172 (20.87)	22 (15.28)
Astrocytoma	22 (33.33)	168 (20.39)	64 (44.44)
Glioblastoma	35 (53.03)	305 (37.01)	48 (33.33)
Oligoastrocytoma	0	113 (13.71)	10 (6.94)
Unknown	0	66 (8.01)	0
Integrated diagnosis			
Oligodendroglioma	8 (12.12)	168 (20.39)	28 (19.44)
Astrocytoma	15 (22.73)	259 (31.43)	26 (18.06)
Glioblastoma	43 (65.15)	397 (48.18)	90 (62.5)
MGMT status			
Methylated	28 (42.42)	529 (64.20)	54 (37.5)
Unmethylated	29 (43.94)	238 (28.88)	34 (23.61)
Missing	9 (13.64)	58 (7.04)	56 (38.89)
Status			
Dead	13 (19.70)	276 (33.50)	68 (47.22)
Alive	11 (16.67)	481 (58.37)	70 (48.61)
Unknown	42 (63.64)	67 (8.13)	6 (4.17)

Abbreviations: O6-methylguanine-DNA methyltransferase, MGMT.

3. Results

3.1. Patient characteristics

The clinical information of 66 patients with adult-type diffuse gliomas was summarized in Table 1. Male-to-female ratio was 1.75:1. The median age at diagnosis was 50 years (range: 25–69 years). Among 66 cases, 9 were diagnosed as oligodendroglioma, 22 as astrocytoma and 35 as GBM based on histopathology. According to the WHO CNS5, 8 cases were diagnosed as *IDH*-mutant, 1p/19q codeleted oligodendroglioma, 15 as *IDH*-mutant astrocytoma, and 43 as *IDH*-WT GBM (Fig. 1). Notably, due to assessment of *IDH* status, diagnoses of 9 *IDH*-WT patients were changed to GBM. In contrast, the diagnosis of one patient harboring *IDH1* R132H was changed to astrocytoma. In addition, one case without 1p/19q codeletion was reconsidered as astrocytoma (Fig. S1A-C).

According to the molecular features including *IDH* and 1p/19q status, oligoastrocytoma was divided into oligodendroglioma, astrocytoma and GBM. As shown in Table 1, the proportion of patients with oligodendroglioma in CGGA and TCGA database was relatively higher. Additionally, lower-grade gliomas evaluated by histology with high-risk markers were corrected as GBM in three cohorts.

3.2. Mutational prevalence across glioma subtypes

High-frequency SNVs in adult-type diffuse glioma were depicted in Fig. 2. *TP53* was the most commonly mutated gene, followed by *TERT*, *IDH1*, *ATRX*, *NF1*, *PTEN*, *TTN*, *EGFR*, *RYR2* and *CIC* (Fig. 2A). *TERTp* was identified in three subtypes, more common in oligodendroglioma (7/8) and GBM (14/43). SNVs in *TTN* and *RYR2* were also found in all subtypes and cohorts. *EGFR* and *CIC* SNVs were mainly detected in GBM and oligodendroglioma (Fig. 2B). *ATRX* and *TP53* mutations were the molecular features of astrocytoma, but their VAFs were insignificant between astrocytoma and GBM (Fig. 2C–D). The mutational burden of high-grade IDH wild-type glioma was significantly higher than that of low-grade IDH wild-type glioma ($p = 0.022$, Fig. S2).

3.3. SNVs, InDels and CNVs in driver genes

Multiple alterations occurred in driver genes *EGFR*, *MET*, *PTEN*, *CDKN2A* and *CDKN2B*. Three patients with GBM harbored both *EGFR* amplification and SNV, and two patients with astrocytoma harbored *MET* SNV and gain (Fig. 3A). Unlike R132 as the predominant variant of *IDH1* in astrocytoma and oligodendroglioma, the alterations in *EGFR* distributed evenly. However, more mutations were in the extracellular domain of *EGFR* rather than the tyrosine kinase domain. Rare *IDH1* R132 L/G and E240Q were also found in astrocytoma (Fig. 3B). Additionally, *TP53* and *ATRX* somatic mutations were also located in the genes, but R273 was relatively high in *TP53* (Fig. S3A, B). *CDKN2A/CDKN2B* homozygous co-deletion occurred in 13 out of 43 cases, but sometimes altered in one of them (Fig. 3C).

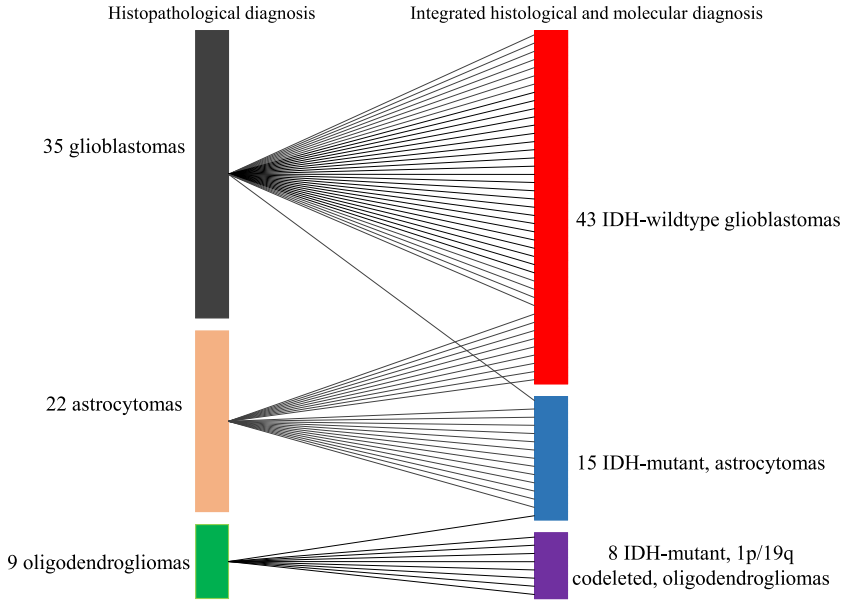

Fig. 1. Schematic illustration of diagnosis changes in 66 patients with adult-type diffuse glioma. Histopathological diagnosis was based on 2016 WHO classification of the CNS tumors (left), and was reclassified based on 2021 WHO classification of the CNS tumors (right).

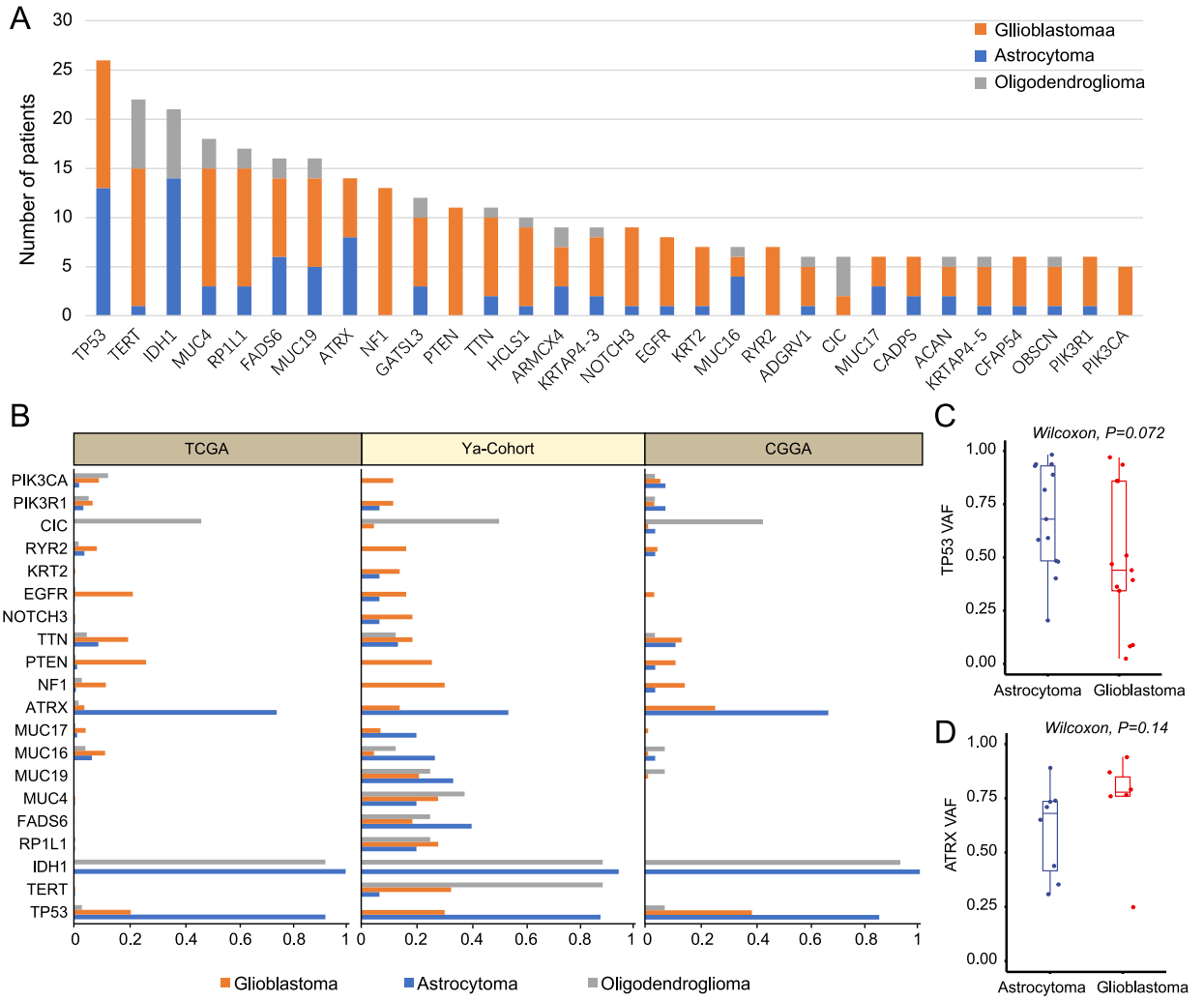

Fig. 2. Mutational prevalence across subtypes. (A) A summary of gene mutations in 66 patients; (B) The mutational frequencies of common genes in our cohort and two public databases; (C) Comparison of the mutational frequency of *TP53* and *ATRX* between astrocytoma and GBM.

3.4. Mutational landscape of key glioma pathways

A list of driver genes was grouped into carcinogenic pathways in the 66 patients (Fig. 4). The alterations in GBM, relatively more malignant type, were distributed in each pathway including p53/RB, DDR, RTK, PI3K, and RAS/MAPK, among which mutations in RTK, PI3K and RAS pathways were most common. Astrocytoma had a more similar mutational landscape to GBM than oligodendroglioma, with numerous mutations in cell cycle and RTK pathways. *TERT*p, *CIC*, *NOTCH1* and MYC alterations were the characteristic markers of oligodendroglioma (Fig. 4).

3.5. Germline variants in DDR pathway

Most germline variants occurred in the genes associated with DDR in cancer. DDR pathway contained multiple repair mechanisms including Fanconi anaemia (FA), homologous recombination (HR), non-homologous end joining (NEJM), base excision repair (BER), direct repair (DR), mismatch repair (MMR), nucleotide excision (NER) and translesion synthesis (TLS). Almost all germline variants were heterozygous except *MSH3* in a patient with GBM (Fig. 5A). *SLX4*, *BRCA2*, *FANCI*, *RECQL4*, *MSH6* and *POLD1* were the genes commonly with germline variants. Three mutations in *BRCA2* including S973L, G2508S and M3234V were evaluated as uncertain significance by InterVar (Fig. S3C). Unlike the somatic variants, germline variants occurred most frequently in oligodendroglioma than GBM and astrocytoma (Fig. 5B). The number of mutations in HRR and FA were all high in three subtypes (Fig. 5C).

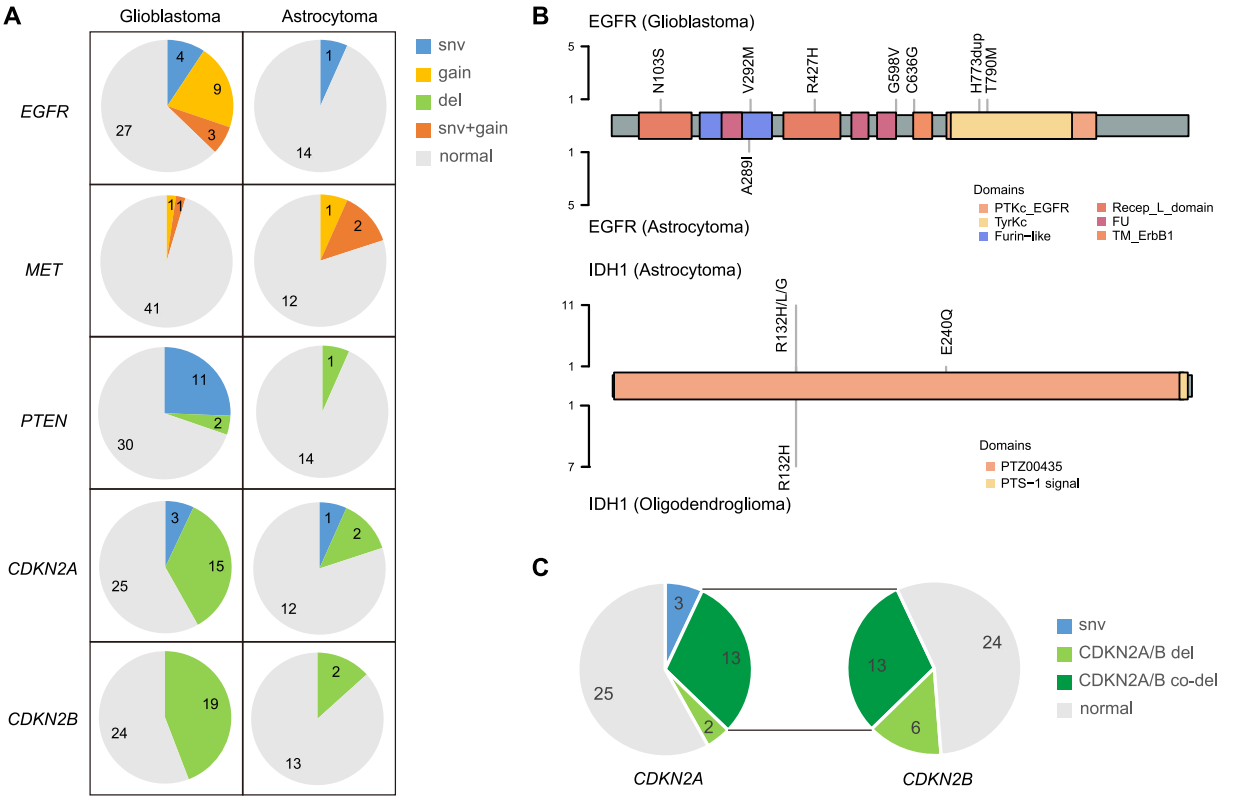

Fig. 3. Multiple types of genetic alterations in adult-type diffuse gliomas. (A) The mutational types of driver genes in GBM and astrocytoma; (B) The spectrum of variants in *EGFR* and *IDH1*; (C) Co-deletion of *CDNKN2A* and *CDNKN2B*.

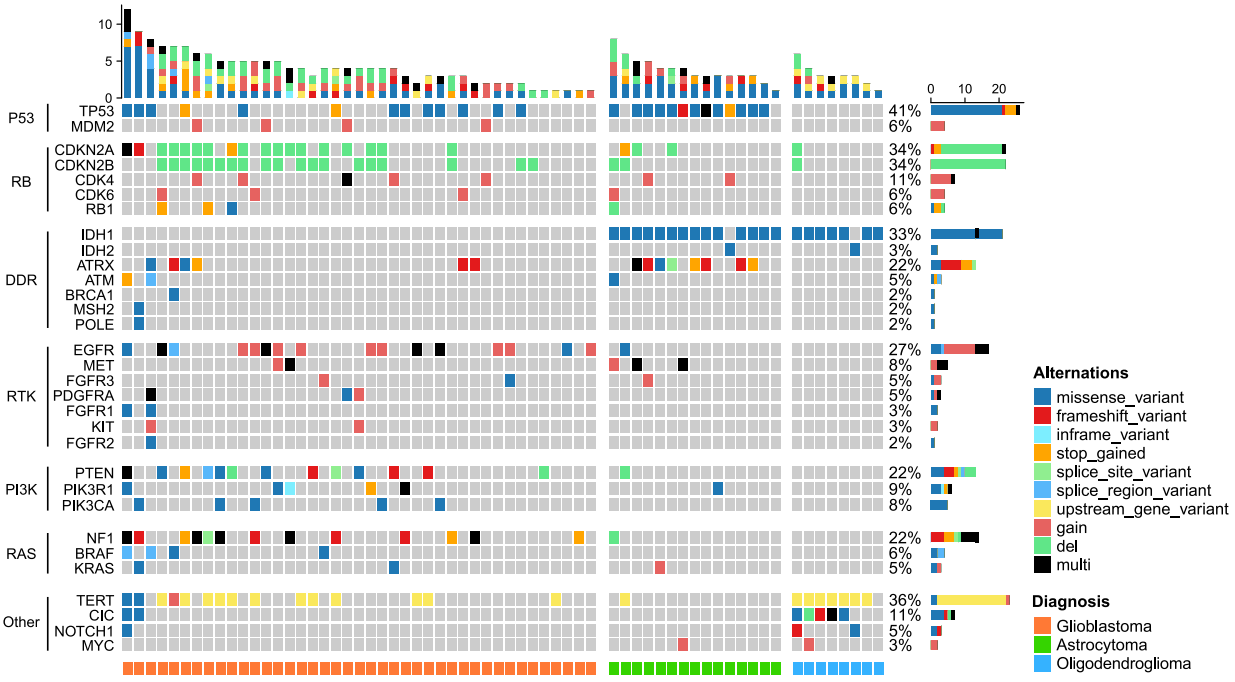

Fig. 4. Genomic landscape of key pathways. A list of driver genes was grouped into the pathways and color coded based on the function of the protein product. Intergraded diagnosis was made at the bottom of the heatmap.

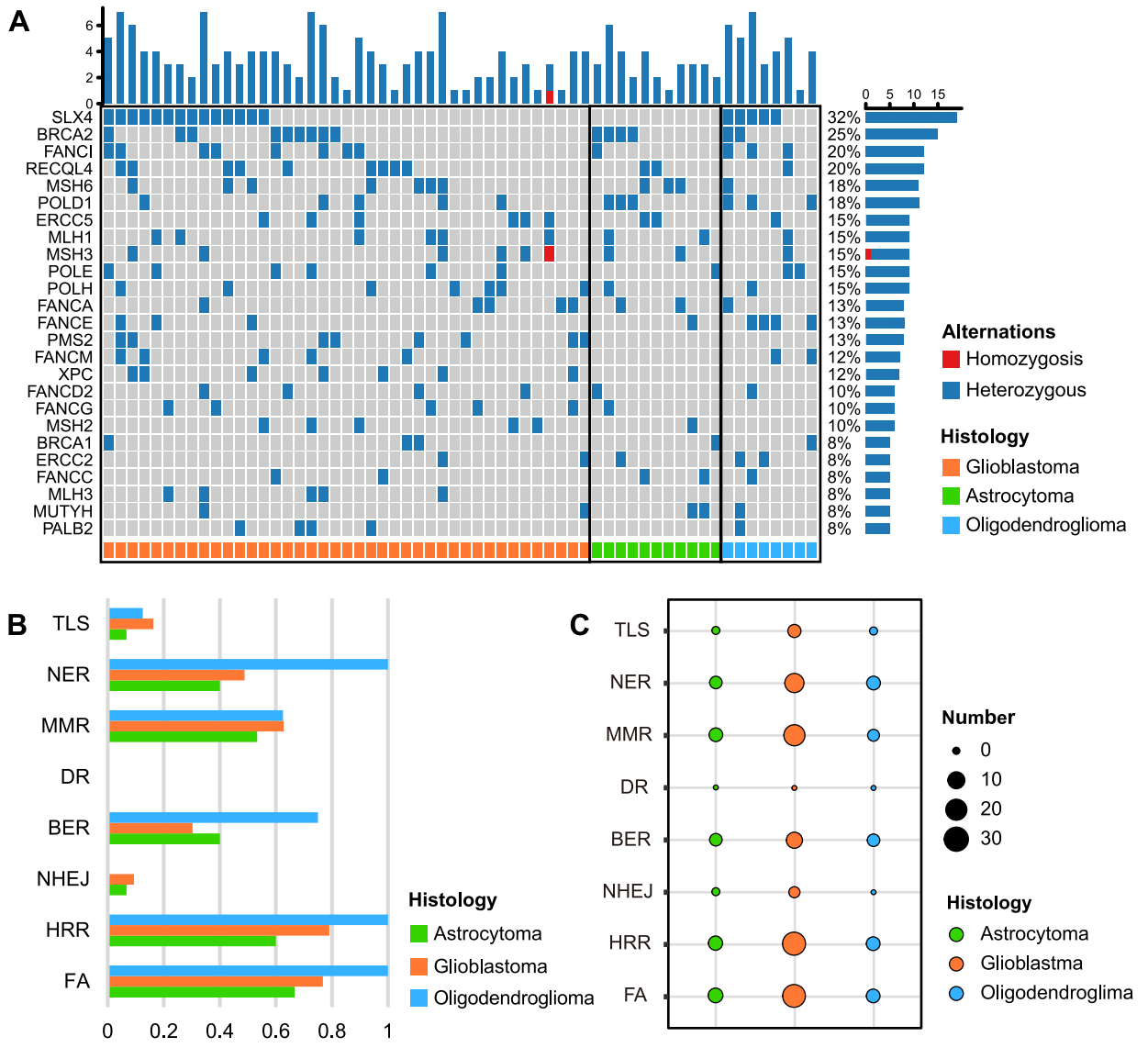

Fig. 5. Germline variants of DDR pathways. (A) Landscape of germline variants in our cohort; (B) Mutational frequencies of DDR genes across three subtypes; (C) The number of mutations in DDR pathways across three subtypes.

3.6. Evaluation of treatment evidence

The genomic aberrations including somatic and germline variants in DDR pathway of adult-type diffuse gliomas were annotated based on the clinical actionability from OncoKB database. There were four therapeutic levels, including FDA-approved drugs, standard care, clinical evidence and biological evidence. Although GBM had 32.6 % of unknown selection, 9.3 % showed the treatment option at level 1 (Fig. 6A and B). High recommendations were from *BRAF*, *ATM*, *EGFR* (Fig. 6C). The level 3 for astrocytoma and oligodendroglioma was *IDH1* inhibitor (Fig. 6C). These actionable molecular targets offered promising options for patients with adult-type diffuse gliomas in further treatment.

3.7. Prognostic biomarkers for IDH-mutant, non-codeleted astrocytoma

IDH was the prognostic marker for all adult-type gliomas. The mutations in key pathways were evaluated for their correlations with prognosis in the three subtypes. PI3K alterations were frequent in GBM, which provided additional treatment options (Fig. 6C). However, *PIK3CA-PIK3R1* mutations were not significant predictive markers for prognosis in GBM. Patients with astrocytoma harboring *PIK3CA-PIK3R1* SNVs had a significantly worse OS in both TCGA and CGGA databases (Fig. 7A and B). Due to the number of missing data of CNVs in CGGA, KM analysis of *CDKN2A/B* deletion and PI3K SNVs was only performed in TCGA. The patients

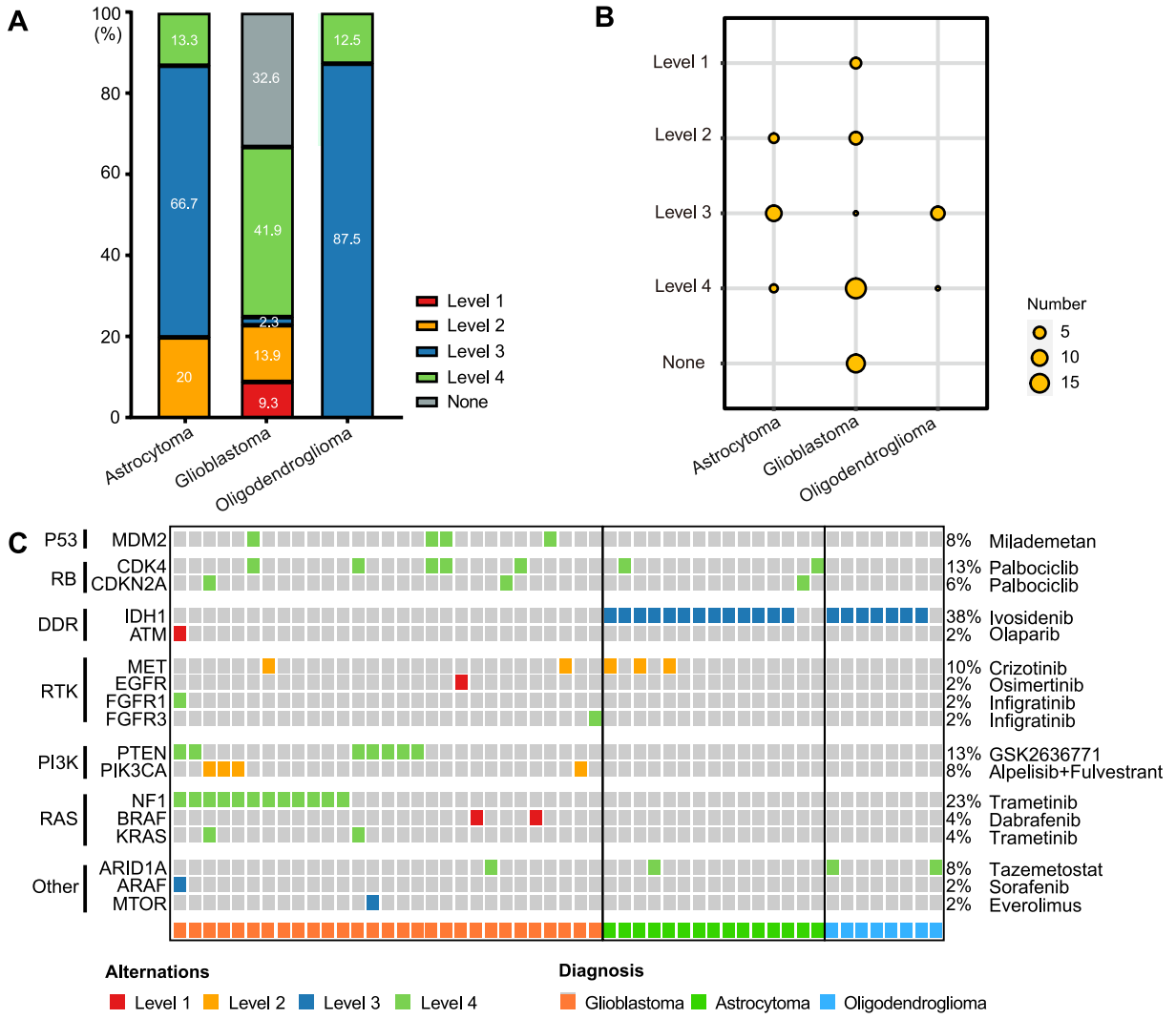

Fig. 6. Levels of treatment options assigned to mutations observed in our cohort according to OncoKB database. The ratio (A) and number (B) of 4 levels in three subtypes; (C) Evidence levels of drugs based on the molecular markers of patients.

harboring both *CDKN2A/B* deletion and *PIK3CA-PIK3R1* showed the worst OS (Fig. 7C). *TERT*p and chr +7/-10 in CGGA, *PIK3CA-PIK3R1*, *EGFR* amplification, chr +7/-10, age, gender and *MGMT* promoter methylation status from TCGA cohort were calculated by multivariate Cox regression. The result suggested *CDKN2A/B* deletion ($p = 0.001$, HR = 4.05, 95 % CI 1.72–9.52) and *PIK3CA-PIK3R1* ($p = 0.003$, HR = 6.1, 95 % CI 1.88–19.86) were the independent prognostic markers of astrocytoma (Table 2). *AEBP1* amplification was significantly associated with worse OS in our GBM cohort ($p = 0.031$) (Fig. 7D) and tended to have a relatively shorter survival ($p = 0.068$) in TCGA cohort (Fig. S4).

4. Discussion

The WHO classification of brain tumors may be further modified with the presence of novel molecular findings. In such a trend of integrated histological and molecular diagnosis in CNS tumors, it is necessary to investigate the effective parameters based on an appropriate detection method. WES is a powerful tool to discover molecular diagnostic and prognostic biomarkers [13]. In this study, WES was used to analyze the genomic profiling of 66 patients with adult-type diffuse glioma. According to the mutational information, 9 cases changed the diagnosis, precise treatment options were provided and *PIK3CA-PIK3R1* SNVs were identified as a prognostic marker of astrocytoma.

Clinically, various methods have been used to test the mutational status. For example, immunohistochemistry is used to detect the specific antibodies of mutations including *IDH1* R132H, *H3* K27M and *BRAF* V600E [19], Sanger sequencing is performed to identify the mutations in tumor DNA. Fluorescence *in situ* hybridization (FISH) is administered to test the chromosome structural variants including chr 1p/19q and chr +7/-10 [20]. WES can integrate the multiple tests to perform a comprehensive genomic analysis for

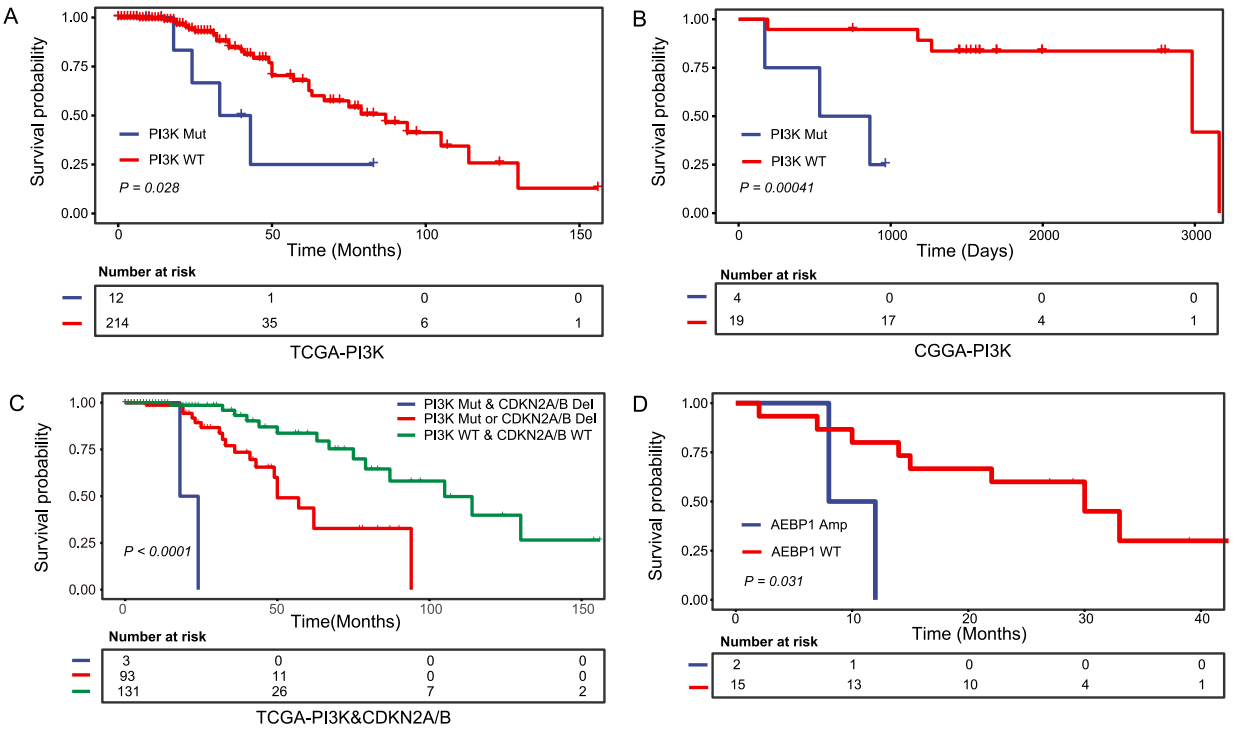

Fig. 7. Prognostic analysis based on Kaplan-Meier method. *PIK3CA-PIK3R*1 mutant patients with astrocytoma showed a significantly worse OS than wildtype patients in TCGA (A) and CGGA (B) databases; (C) Patients with astrocytoma harboring both *PIK3CA-PIK3R*1 and *CDKN2A/B* deletion had the worst OS, while the OS of patients with double wildtype was best; (D) GBM patients with *AEBP1* amplification revealed a shorter OS than wildtype patients in our cohort.

Table 2
Multivariate Cox regression of clinical and molecular information in patients with astrocytoma from the TCGA.

Variables	*P*-value	HR (95 % CI)
Age≥60 years	0.916	0.93 (0.258–3.376)
Male	0.863	0.94 (0.457–1.928)
MGMT promoter unmethylated	0.727	0.82 (0.263–2.537)
chr +7/-10	0.998	2.61E-6 (0-Inf)
EGFR amplification	0.763	0.88 (0.399–1.963)
CDKN2A/B deletion	0.001	4.05 (1.72–9.52)
PIK3CA-PIK3R1	0.003	6.1 (1.88–19.86)

patients with adult-type diffuse gliomas.

IDH status and 1p/19q co-deletion were used to classify oligodendroglioma, astrocytoma and GBM defined by histology [6]. Astrocytoma or oligodendroglioma without *IDH1/2* mutations was reclassified as *IDH*-WT, GBM. Therefore, more GBM patients were defined in all three cohorts. A case harboring 1p36/19q deletion, *ATRX* and *TP53* mutations diagnosed as oligodendroglioma according to histology was changed to astrocytoma. ATRX status was thought to have a higher diagnostic priority than 1p/19q co-deletion, 1p/19q co-deletion oligodendroglioma with ATRX absent would be considered as astrocytoma [12]. In addition, 1p36/19q deletion identified by FISH was not equal to 1p/19q co-deletion, which was not detected by NGS. The differential diagnosis of astrocytoma and oligodendroglioma based on molecular parameters still need to be explored.

Our results demonstrated the high mutational frequencies of *TP53*, *ATRX*, *NF1*, *PTEN*, *TTN* and *EGFR* and molecular features across three subtypes, which were consistent with previous studies [21–23]. *TERT*p in our cohort was detected using WES, which was different from TCGA and CGGA cohorts. Passenger mutations, such as *TTN* and *RYR2* [21], were discovered frequently and their function in cancer might be identified including addition of tumor mutational burden. Moreover, we also identified relatively higher mutational frequencies of *MUC16*, *MUC17* and *MUC19* in our cohort. MUC family mutations occurred commonly in cancer, *MUC16* tended to be a favorable prognostic factor due to high immune infiltration in H3 G34-mutant diffuse hemispheric glioma [24].

EGFR alterations in GBM included multiple patterns, such as SNVs, CNVs, and fusions [25]. The *EGFR*-targeted drugs mainly fell into two categories: tyrosine kinase inhibitors (TKIs), such as gefitinib and erlotinib, and monoclonal antibodies (mAb) including cetuximab and nimotuzumab [26]. Currently, no TKIs have been approved for patients with GBM [27], and less mutations in *EGFR* and

PDGFRA were located at the intracellular domain. Therefore, mAb targeted to the extracellular domain might be more promising. WES contributed to revising the diagnosis by detecting rare SNVs such as R132 L/G, E240Q of *IDH1*. In our study, *CDKN2A/CDKN2B* homozygous co-deletion occurred in 13 out of 43 cases. There is a study suggesting that homozygous co-deletion of *CDKN2A* and *CDKN2B* frequently occurs in malignant gliomas [28].

In our study, GBM showed the highest mutational heterogeneity in the adult-type diffuse gliomas, with aberrations mainly converging on key pathways including cell cycle, RTK, PI3K, RAS and DDR. Different from oligodendroglioma, astrocytoma basically had less alterations than GBM in such pathways. Telomere maintenance was required in proliferation of glioma, thus *TERT*p was universal in GBM [29]. *TERT*p was also the molecular feature of oligodendroglioma, and showed significant mutual exclusivity with both *TP53* and *ATRX*, which was a characteristic of astrocytoma. The VAF of *ATRX* was insignificant between astrocytoma and GBM, but astrocytoma tended to have a relatively high VAF of *TP53* than GBM. Oligodendroglioma had the highest frequency of germline variants in DDR pathway, especially in NER, BER, HRR and FA. These molecular differences across three subtypes may provide some references for differential diagnosis and targeted intervention.

Compared with more complicated regulation pathways like cell cycle and PI3K, RTK and DDR pathways presented more actionable targeted markers. *ATM* mutations, germline and somatic variants of *BRCA2* in HRR pathway could remind the patients of benefiting from PARPi [30,31]. RTK aberrations including *EGFR* and *MET* provided the options of TKIs with ability in blood-brain barrier for patients [32,33]. Currently, FDA has approved Tafinlar plus Mekinist for patients with solid tumors harboring *BRAF* V600E [34]. To some extent, it offers a new selection for the subsequent therapy of two patients with *BRAF* V600E in our cohort.

Regarding the prognostic factors of gliomas, *IDH1/2*, 1p/19q codeletion, *ATRX*, *BRAF* were all confirmed to be the positive prognostic markers, while *TERT*p, chr +7/-10, *EGFR* amplification were all negative predictors [7]. In our study, patients with astrocytoma harboring *PIK3CA* or *PIK3R1* mutation showed a significantly worse OS than those with WT in both TCGA and CGGA cohorts. Survival analysis suggested *PIK3CA-PIK3R1* and heterozygous *CDKN2A/B* deletion were the independent prognostic factor of astrocytoma, which was proved by a previous study [12]. In cIMPACT-NOW Update 3, diffuse astrocytic glioma with molecular features of GBM, *TERT*p, chr +7/-10 and *EGFR* amplification, was considered as WHO grade IV [11]. Aberrant PI3K signaling is associated with more than 80 % of GBM cases [35]. Therefore, *PIK3CA-PIK3R1* mutations were also negative prognostic markers of diffuse astrocytic glioma. The follow-up of two astrocytoma cases with *PI3K* mutations in our cohort is in progress, and larger samples are needed to validate the hypothesis. AEBP1 could promote glioma cell proliferation and invasion, and its overexpression was correlated with short survival of GBM patients [36,37]. In our study, *AEBP1* amplification was identified to be significantly associated with worse OS in GBM, suggesting that *AEBP1* amplification might be a prognostic marker for GBM.

The current study may still benefit from a larger sample size for a more comprehensive research into mutations and SNVs of glioma and a more accurate survival analysis in glioma patients. Functional validations of the identified biomarkers and predicted drugs in the study need to be performed in larger cohorts for a practical realization of the manuscript.

5. Conclusions

We comprehensively characterized the mutational landscape of adult-type diffuse gliomas, and identified the differential diagnostic, therapeutic, and prognostic biomarkers for patients. This analysis may provide new insights into the genesis, classification, precise treatment and prognosis of glioma. In vivo validations of the identified biomarkers and evaluations of predicted drugs may prove valuable for future studies of precision medicine in glioma treatment.

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Data availability statement

The data used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Patients included in our study provided informed consent, and they consented to participate in non-routine care procedures such as next generation sequencing. Collection and analysis of the samples were approved by the Institutional Review Board of Xiangya Hospital Central South University (2024040377).

Consent for publication

All authors give full consent for publication.

CRediT authorship contribution statement

Zhenyan Li: Writing – original draft, Formal analysis, Conceptualization. **Zhenghao Deng:** Investigation, Formal analysis, Data

curation. **Fangkun Liu:** Investigation, Formal analysis, Data curation. **Chuntao Li:** Investigation, Data curation. **Kui Yang:** Investigation, Data curation. **Xuan Gong:** Investigation, Data curation. **Songshan Feng:** Investigation, Data curation. **Yu Zeng:** Investigation, Data curation. **Hongshu Zhou:** Investigation, Data curation. **Fan Fan:** Investigation, Data curation. **Chengke Luo:** Investigation, Data curation. **Zhixiong Liu:** Writing – review & editing, Supervision. **Mingyu Zhang:** Writing – review & editing, Project administration, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Not applicable.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e37712.

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