Total copy number variation as a prognostic factor in adult astrocytoma subtypes

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Abstract
Since the discovery that IDH1/2 mutations confer a significantly better prognosis in astrocytomas, much work has been done to identify other molecular signatures to help further stratify lower-grade astrocytomas and glioblastomas, with the goal of accurately predicting clinical outcome and identifying potentially targetable mutations. In the present study, we subclassify 135 astrocytomas (67 IDH-wildtype and 68 IDH-mutant) from The Cancer Genome Atlas dataset (TCGA) on the basis of grade, IDH-status, and the previously established prognostic factors, CDK4 amplification and CDKN2A/B deletion, within the IDH-mutant groups. We analyzed these groups for total copy number variation (CNV), total mutation burden, chromothripsis, specific mutations, and amplifications/deletions of specific genes/chromosomal regions. Herein, we demonstrate that across all of these tumor groups, total CNV level is a relatively consistent prognostic factor. We also identified a trend towards increased levels of chromothripsis in tumors with lower progression-free survival (PFS) and overall survival (OS) intervals. While no significant differences were identified in overall mutation load, we did identify a significantly higher number of cases with mutations in genes with functions related to maintaining genomic stability in groups with higher mean CNV and worse PFS and OS intervals, particularly in the IDH-mutant groups. Our data further support the case for total CNV level as a potential prognostic factor in astrocytomas, and suggest mutations in genes responsible for overall genomic instability as a possible underlying mechanism for some astrocytomas with poor clinical outcome.

Keywords: Copy number variation, CNV, Astrocytoma, Glioma, Glioblastoma, GBM, TCGA

Introduction
Diffuse gliomas are among the most common primary CNS tumors, representing approximately 27% of all primary brain tumors [29, 30]. Due to their infiltrative nature, these tumors are surgically incurable, although the exact prognosis depends on numerous histologic and molecular factors. The standard of care now dictates molecular classification of gliomas based on IDH1/2 mutation status as IDH-mutant gliomas have a significantly better prognosis than their IDH-wildtype grade-matched counterparts [25]. While histologic grade shows correlation with overall survival within these molecular groups, there remains significant heterogeneity in clinical outcome.

Since the widespread adoption of the 2016 WHO classification system, much work has been done to find further molecular markers to sub-stratify both IDH-mutant and IDH-wildtype astrocytomas in hopes of better predicting tumor behavior and outcome, including identification of secondary mutations, focal genetic alterations, methylation patterns, and multivariate prognostic models [3, 24, 42, 44]. Within the IDH-wildtype groups, these studies have suggested that lower-grade gliomas (LGG) with EGFR amplification, gain of chromosome 7 and loss of 10, or TERT promoter mutations will have aggressive clinical courses and outcomes similar to IDH-wildtype glioblastoma, regardless of histologic features. In IDH-mutant groups, lower-grade tumors with alterations in genes in the retinoblastoma pathway, including
amplification of CDK4 and deletion of CDKN2A/B, demonstrate significantly worse clinical behavior and shorter patient survival [1, 5, 8, 33].

Previous work has demonstrated that IDH-mutant glioblastomas have higher levels of total copy number variation (CNV) across the entire genome and evidence of more frequent chromothripsis than lower-grade IDH-mutant astrocytomas [9]. We subsequently showed that in IDH-mutant grade II and III astrocytomas, this increased level of CNV was present before progression to glioblastoma in cases with exceptionally poor outcomes, defined by rapid progression to glioblastoma and short survival times after initial diagnosis [36, 37]. The poor outcome appeared to be directly correlated with overall CNV, but not other factors, including mutation burden or differences in methylation profiles, suggesting that this large scale CNV pattern could potentially override the beneficial effect of IDH-mutant status.

To better understand the effect of CNV, we analyzed 135 astrocytic tumors from The Cancer Genome Atlas (TCGA) (67 IDH-wildtype and 68 IDH-mutant cases) with respect to clinical outcome, CNV levels, chromosomal and specific gene amplification and deletion events, chromothripsis, total mutation load, specific mutations in known glioma/GBM genes, and mutations in genes associated with overall genomic instability. Building on our previous results, we performed wide scale genomic analysis, on a framework of pre-established prognostic factors including grade, IDH1/2-status, and the presence of CDK4 amplifications or CDKN2A/B deletions. With the exception of 2 IDH1/2-wildtype cases, CDK4 amplification and CDKN2A/B deletion were found to be mutually exclusive. We divided the cases into 5 groups: IDH1/2-mutant LGG without CDK4 amplification or CDKN2A/B deletion (Group 1), IDH1/2-mutant LGG with either CDK4 amplification or CDKN2A/B deletion LGG (Group 2), IDH1/2-mutant GBM (Group 3), IDH1/2-wildtype LGG (Group 4), and IDH1/2-wildtype GBM (Group 5).

We demonstrate that higher levels of CNV and chromothripsis are correlated with clinical outcome in the IDH-mutant groups, while the IDH-wildtype groups had uniformly high CNV levels and poor outcomes. Other prognostic factors appear to be inconsistent. We also identified a significantly higher number of mutations in genes involved with overall genomic stability, paralleling levels of overall CNV and chromothripsis, in the cases with worse prognosis. While defining the exact role of genes involved in progression may still be needed for development of individualized targeted therapies, use of CNV could potentially serve as a clinically impactful model for prognostication of different astrocytoma subtypes, and may aid in our understanding of the underlying biology of these tumor types.

Methods

TCGA case selection

Using the cBioportal interface, we performed a search of 380 glioblastoma cases and 539 lower-grade gliomas (LGG, defined here as WHO grade II-III) [6, 9, 14]. The original histologic diagnoses reported in TCGA included astrocytoma, oligoastrocytoma, anaplastic astrocytoma, anaplastic oligoastrocytoma, and glioblastoma. All cases were manually reclassified according the WHO 2016 criteria as diffuse astrocytomas (WHO grade II-IV) by histology, intact 1p/19q status, and IDH1/2, ATRX, and TP53 status. Oligodendrogliomas were specifically excluded on the basis of 1p/19q co-deletion, as these tumors have been shown to have different underlying molecular drivers and a more favorable clinical outcome as a group. All cases selected represented the first resection specimen and were segregated into lower-grade (WHO grades II and III) [9, 28, 35] and GBM (WHO grade IV) within the IDH1/2 mutation groups. We identified 5 groups based on previously identified prognostic factors, including histologic grade, IDH1/2, CDK4, and CDKN2A/B status [1, 8, 25, 31, 45] and selected groups of TCGA cases that met these criteria: Group 1, IDH1/2-mutant LGG without CDK4 amplification or CDKN2A/B deletion (n = 24, mean age = 38.8 ± 1.9 years); Group 2, IDH1/2-mutant, CDK4-amplified/CDKN2A/B-deleted LGG (n = 22, mean age = 38.8 ± 1.9 years); Group 3, IDH1/2-mutant GBM (n = 22, mean age = 40.5 ± 2.7 years); Group 4, IDH1/2-wildtype LGG (n = 25, mean age = 54.0 ± 2.6 years); Group 5, IDH1/2-wildtype GBM (n = 42, mean age = 62.8 ± 1.7 years) (Table 1).

Genetic and epigenetic analysis

The gene expression (Illumina HiSeq, RNASeq) and DNA methylation data (Illumina Human Methylaton 450) was downloaded for the selected TCGA cases and analyzed with TCGAbiolinks [10]. The Affymetrix SNP 6.0 microarray data normalized to germline for copy number analysis for the same TCGA cases was downloaded from Broad GDAC Firehose (http://gdac.broadinstitute.org/runs/stddata__2016_01_28/). The fraction of copy number alterations was calculated from the above data as the fraction of the genome with log2 of copy number > 0.3 following the procedure used in cBioportal [14]. The mutation load is the number of nonsynonymous mutations seen in a sample. The differential analysis and visualization of mutations was done using Maftools [26]. The ideogram for visualization of genome-wide copy number variation results was generated using Genome Decoration Page (https://www.ncbi.nlm.nih.gov/genome/tools/gdp). The pathway and network analyses were conducted using Qiagen’s IPA tool (www.qiagen.com/ingenuity) and R 3.4.1 (http://www.R-project.org/).
Table 1 Summary of available clinical, histologic, and molecular data from each astrocytoma subgroup analyzed

| Group | Tumor Type     | n  | Age at Onset (years) | Median Progression-Free Survival (months) | Median Overall Survival (months) | Histologic Grade (II/III/IV) | CNV Level (%) | Chromothripsis | Cases with Chromothripsis | Mutation Count | Instability Gene Mutations |
|-------|----------------|----|----------------------|------------------------------------------|--------------------------------|-------------------------------|----------------|---------------|--------------------------|----------------|--------------------------|
| 1     | IDH-mut LGG    | 24 | 38.8 ± 1.9           | 95                                       | > 172                          | 12/12/0                       | 9.1 ± 1.6 | 2 (8.3%)     | 43 ± 10.5                | 1 (4.1%)       |
| 2     | IDH-mut CDK4/  |     |                      |                                          |                               |                               |               |              |                          |                |                          |
|       | CDKN2A/B LGG   | 22 | 38.8 ± 1.9           | 36                                       | 4/18/0                        | 21.3 ± 2.5                    | 6 (27.3%) | 33.3 ± 1.3 | 7 (31.8%)                |                 |                          |
| 3     | IDH-mut GBM    | 22 | 40.5 ± 2.7           | 33                                       | 0/0/22                        | 20 ± 2.7                      | 9 (40.9%) | 67.4 ± 2.7 | 8 (36.4%)                |                 |                          |
| 4     | IDH-wt LGG     | 25 | 54.0 ± 2.6           | 10.5                                     | 0/25/0                        | 19.9 ± 1.8                    | 5 (20.0%) | 64.9 ± 16.7 | 5 (20.0%)                |                 |                          |
| 5     | IDH-wt GBM     | 42 | 62.8 ± 1.7           | 6                                        | 0/0/42                        | 22.2 ± 1.6                    | 11 (26.2%) | 57.0 ± 2.5 | 10 (23.8%)               |                 |                          |

GISTIC analysis
The GISTIC (Genomic Identification of Significant Targets in Cancer) 2.0 algorithm was used to identify regions of the genome that are significantly amplified or deleted between the 5 groups of IDH1/2-mutant and wildtype astrocytoma cases [27]. Each area of CNV is assigned a G-score that considers the amplitude of the alteration as well as the frequency of its occurrence across samples. The false discovery rate (FDR) was then used to determine the relative significance of each abnormality. Each region predicted to be significantly different between the 5 groups was screened for tumor suppressor genes, oncogenes, and other genes associated with glioma and malignancy [2, 27]. GISTIC 2.0 analysis was run using GenePattern [32].

Mutation analysis of genes involved in maintenance of genomic stability
A group of genes with previously identified roles in cell proliferation and maintaining chromosomal stability were identified by a literature review and included the following genes: APC, ATM, ATR, BLM, BRCA1 (FANCs), BRCA2 (FANCd1), BUB1B, CHK1, CLSPN, DNA-PK (PRKDC), EME1, FANCA, FANCB, FANCC, FANCd2, FANCE, FANCf, FANCg, FANCi, FANCj (BRIP1), FANCI, FANCm, FANCN (PALB2), FANCO (RAD51C), FANCP (SLX4), FANCQ, FANCr, FANCt (UBE2T), HUS1, LIG4, MUS81, NBN, POLK, POLN, RAD51, RAD52, REV3, SMC1, SMC1B, TOP1, TP53, WRN, and XLF [7, 16, 36]. Variant annotation was performed using COSMIC [13], dbSNP [39], ClinVar [22], CanProVar 2.0 [23], The 1000 Genomes Project [15], and FATHMM-MKL [40].

Statistical analysis
Differences in patient age, mutation burden, and CNV were evaluated using Analysis of Variance (ANOVA). Significance of survival curves were calculated using the Mantel-Cox test (Log-rank test). Proportion of cases with chromothripsis and mutations specifically associated with genome instability were calculated using Fisher’s Exact test. Coefficients of variation (CNV vs survival times) were calculated using Pearson correlation coefficient. All statistical calculations were performed with GraphPad Prism version 7.04 (GraphPad, La Jolla, CA).

Results
Clinical characteristics
As previously demonstrated [1, 8], IDH-mutant LGGs (group 1) had a significantly longer progression-free survival (PFS; median 95 months) and overall survival (OS; > 172 months) than IDH-mutant LGGs with CDK4 amplifications or CDKN2A/B deletions (group 2) (PFS 32 months, \( p = 0.0224 \); OS 36 months, \( p = 0.0150 \)) and a significantly longer PFS and OS than IDH-mutant GBM (group 3) (PFS 10 months, \( p = 0.0032 \); OS 33 months, \( p = 0.0081 \)). A significant difference was not found between IDH-mutant LGGs with CDK4 amplifications or CDKN2A/B deletions (group 2) and IDH-mutant GBM (group 3) in terms of PFS (\( p = 0.0769 \)) or OS (\( p = 0.2892 \)) (Fig. 1a-b). No significant difference was found between IDH-wildtype LGGs (group 4) and IDH-wildtype GBM (group 5) in terms of PFS (\( p = 0.2050 \)) or OS (\( p = 0.9351 \)) (Fig. 1c-d). Amplifications in CDK4 and deletions in CDKN2A/B did not have prognostic significance within the IDH-mutant GBM group in terms of PFS (\( p = 0.8406 \)) or OS (\( p = 0.1471 \)) (Fig. 2a-b).

No significant difference was identified in the median age of onset within the IDH-mutant groups 1–3, however there was a significant difference between the average age of onset in IDH-mutant LGG cases (38.8 ± 1.9 years) and IDH-wildtype LGG cases (54.0 ± 2.6 years) (\( p < 0.0001 \)). There was also a significant difference in age of onset between IDH-wildtype LGGs (54.0 ± 2.6 years) and IDH-wildtype GBMs (62.8 ± 1.7 years) (\( p = 0.0047 \)). There was a trend toward higher histologic tumor grade identified between groups 1 and 2. All IDH1/2-wildtype LGG tumors (group 4) were WHO grade III by histology at initial diagnosis (Table 1).

Total copy number analysis differences
Mirroring the difference in clinical outcome, the total percentage of the genome with copy number alterations was low in the LGGs without CDK4 or CDKN2A/B alterations and uniformly high in the other 4 groups.
Table 1. Total copy number variation was 9.1 ± 1.6% in IDH-mutant LGGs (group 1), a significantly lower level than IDH-mutant LGGs with CDK4 or CDKN2A/B alterations (p = 0.0224) and IDH-mutant GBMs (p = 0.0032), but not between IDH-mutant LGGs with CDK4 or CDKN2A/B alterations and IDH-mutant GBMs (p = 0.0769) in terms of progression-free survival (a). There was also a significant difference between IDH-mutant LGGs and both IDH-mutant LGGs with CDK4 or CDKN2A/B alterations (p = 0.0150) and IDH-mutant GBMs (p = 0.0081), but not between IDH-mutant LGGs with CDK4 or CDKN2A/B alterations and IDH-mutant GBMs (p = 0.2892) in terms of overall survival (b). No significant differences are identified between IDH-wildtype LGGs and IDH-wildtype GBMs in terms of progression-free survival (p = 0.2050) (c) or overall survival (p = 0.9351) (d).

Chromosomal analysis and GISTIC
Analysis of the IDH-mutant tumors (groups 1–3) revealed a heterogeneous assortment of genomic alterations with few consistent chromosomal regions with amplifications or deletions, although there is a clear increase in number.
of overall alterations between the group 1 IDH-mutant LGGs and the group 2 IDH-mutant LGGs with CDK4 amplification/CDKN2A/B deletion and group 3 IDH-mutant GBM (Fig. 5), quantified in Fig. 3a. Conversely, IDH-wildtype LGGs and GBMs form a relatively homogeneous group with consistent amplifications, including large amplifications along chromosome 7, deletions on 9p, and deletions of chromosome 10 (Fig. 6).

As expected based on our case selection, Genomic Identification of Significant Targets In Cancer (GISTIC) analysis showed high levels of amplification of 12q14.1 (a region containing CDK4) in all gliomas with poor prognosis (i.e., groups 2, 3, 4, and 5) but not in group 1. Similarly, 9p21.3 (a region containing CDKN2A) showed frequent deletions in groups 2, 3, 4, and 5 but not in group 1. IDH-wildtype tumors had consistent amplifications of 7p11.2 (containing EGFR) and 1q32.1 and deletions of 1p32.3, but only IDH-wildtype GBM had consistent deletions at 10q23.31. Interestingly, IDH-mutant GBM and IDH-mutant LGGs with CDK4 amplification/CDKN2A/B deletion both had amplifications at 2p24.3 (a chromosomal region containing MYCN). This was not identified in IDH-mutant LGGs with a good clinical outcome or in the IDH-wildtype tumors. Group 1 IDH-mutant LGGs had significant consistent amplifications at 3p25.2, 5q31.1, 8q24.13, 11q24.2, 13q34, 19q13.12, Xp22.32, and Xq28, as well as consistent deletions at 3p14.1, 9p24.2, 11p12, 13q14.3, 14q24.3, and Xq21.1 that were not identified in any other tumor group (Fig. 7). All cytobands shown met the criterion of false discovery rate (FDR) ≤0.25. The annotated cytobands met the criterion of FDR ≤0.05.
Amplifications and deletions in specific genes of interest were rare in the group 1 IDH-mutant LGGs, per our study design (Additional file 1: Figure S1). IDH-mutant astrocytomas with poor clinical outcomes (groups 2 and 3) also showed more frequent amplifications of GLI1, KIT, KDR, MYC, MYCN, GATA3, CCND2, and KRAS as well as more frequent deletions of PTEN, PTPRD, ATRX, and RB1 (Additional file 2: Figure S2 and Additional file 3: Figure S3). IDH-wildtype groups frequently had amplifications in EGFR, PDGFRA, CDK4, MDM2, MDM4, KIT, and KDR, as well as deletions in CDKN2A/B, and PTEN. CDK4 amplification and CDKN2A/B deletion appear to be almost mutually exclusive, as they only occur together in one IDH-wildtype LGG case and one IDH-wildtype GBM case (2.3% of cases with these alterations) (Additional file 4: Figure S4 and Additional file 5: Figure S5).

Analysis of chromothripsis
Chromothripsis, defined here as 10 or more alternating bands of amplifications and deletions in a single chromosome [9, 21], was identified in at least one tumor in each of the 5 groups analyzed (Table 1). Comparing individual groups, there was a significant difference in the number of cases with chromothripsis between group 1 LGGs without CDK4 amplification or CDKN2A/B deletion and both IDH-mutant LGGs with CDK4 or CDKN2A/B alterations ($p = 0.0003$) and IDH-mutant GBMs ($p = 0.0078$), but not between IDH-mutant LGGs with CDK4 or CDKN2A/B alterations and IDH-mutant GBMs ($p = 0.7783$) (a); no significant difference was found in total mutation burden between any group of IDH-mutant astrocytoma (b). There was no significant difference between IDH-wildtype LGGs and IDH-wildtype GBMs in terms of overall copy number variation ($p = 0.3732$) (c) or total mutation burden ($p = 0.5627$) (d).
compared to all IDH-mutant tumors with poor prognosis (groups 2 and 3 combined) ($p = 0.0211$). No significant difference was observed between groups 2 and 3 ($p = 0.3475$) or between the IDH-wildtype groups 4 and 5 ($p = 0.7681$) (Fig. 8a).

**Mutation analysis**

Overall mutation load did not differ significantly between any of the tumor groups analyzed (group 1 vs group 2, $p = 0.3863$; group 1 vs group 3, $p = 0.2745$; group 2 vs group 3, $p = 0.2728$; group 3 vs group 5, $p = 0.3318$; or group 4 vs group 5, $p = 0.5627$) (Fig. 3b, d).

Analysis of individual genes in the IDH-mutant groups reveals consistently high rates of $TP53$ mutations in all 3 groups (91–100% of cases) and relatively high rates of $ATRX$ mutations (68–77% of cases). There are other scattered pathogenic mutations, with elevated numbers of $EGFR$ (14%) and $PIK3R1$ (27%) mutations in the IDH-mutant GBM group (Additional file 1: Figure S1, Additional file 2: Figure S2 and Additional file 3: Figure S3).

The IDH-wildtype tumor groups have significantly lower rates of $ATRX$ mutation in both the LGG group (4%) and GBM group (0%), as well as lower rates of $TP53$ mutations in the LGG group (20%) and GBM

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**Fig. 4** Scatter plots of copy number variation (%) plotted against survival time (months) in grouped IDH-mutant LGGs and IDH-mutant GBMs with Pearson’s R values, illustrating significant inverse correlations between the two data points in terms of (a) progression-free survival ($r = -0.3415$; $p = 0.0047$) and (b) overall survival ($r = -0.3098$; $p = 0.0102$)

**Fig. 5** Overall amplification and deletion levels and chromosomal locations in IDH-mutant LGGs without CDK4 amplification or CDKN2A/B deletion (a), IDH-mutant LGGs with either CDK4 amplification or CDKN2A/B deletion (b), and IDH-mutant GBMs (c)
group (33%). Mutations in \textit{EGFR} (32\% in LGG; 24\% in GBM), \textit{PTEN} (28\% in LGG; 31\% in GBM), \textit{NF1} (32\% in LGG; 7\% in GBM), and \textit{RB1} (12\% in LGG; 12\% in GBM) were seen significantly more frequently in these tumors than in the \textit{IDH}-mutant groups 1–3 (Additional file 4: Figure S4 and Additional file 5: Figure S5).

**Mutation analysis of genes associated with overall genomic instability**

Using a 43-gene panel of genes known to be associated with chromosomal instability (excluding \textit{TP53} due to its relative frequency across all groups), we detected a significant difference in the number of mutations between group 1 \textit{IDH}-mutant LGGs without \textit{CDK4} amplifications or \textit{CDKN2A/B} deletions and group 2 \textit{IDH}-mutant LGGs with either alteration (\(p = 0.0197\)) as well as between group 1 \textit{IDH}-mutant LGGs and group 3 \textit{IDH}-mutant GBMs (\(p = 0.0086\)) (Fig. 8b). No significant difference was identified between the two groups of \textit{IDH}-wildtype astrocytomas (\(p = 0.5443\)). No significant difference was identified between \textit{IDH}-mutant tumors with poor outcomes (group 2 + 3) and \textit{IDH}-wildtype tumors with poor prognosis (group 4 + 5) (\(p = 0.1297\)), although there was a trend toward fewer mutations in genes specifically associated with chromosomal instability in the \textit{IDH}-wildtype groups (Tables 1 and 2). These data mirror the trend in level of total CNV and chromothripsis identified in each tumor group.

**Discussion**

Diffuse gliomas represent approximately 27\% of all primary brain tumors and approximately 81\% of all malignant brain tumors [29, 30], making them an intense subject of study and public health expenditure. The recent changes to glioma classification in the 2016 WHO classification system are based around the beneficial role of \textit{IDH}-mutation in gliomas [25]; however, significant molecular heterogeneity exists within the lower-grade/\textit{IDH}-mutant and wildtype gliomas. More work is necessary to further stratify \textit{IDH}-mutant astrocytomas [44], and there is evidence that many \textit{IDH1/2}-wildtype LGGs may be biologically identical to \textit{IDH1/2}-wildtype glioblastomas [17, 34]. In addition, new methods to analyze whole genome genetic and epigenetic signatures are leading to new definitions for many of these tumor groups with significant prognostic implications [4, 38, 43].

We previously reported that increased CNV is associated with a more aggressive biological behavior and poor overall survival in \textit{IDH}-mutant LGGs [36, 37]. With whole genome analysis in the current study, we show...
that CNV correlates with clinical outcome, and was significantly lower in the IDH-mutant LGGs compared to the IDH-mutant LGGs with CDK4 or CDKN2A/B alterations or IDH-mutant GBMs. (Figs. 3a and 4). These results confirm our previous findings, in which IDH-mutant LGG cases selected solely on the basis of poor clinical outcome displayed significantly higher levels of CNV before progression to GBM than a cohort with more conventional progression-free and overall survival [36]. The elevated CNV levels in IDH-mutant LGGs with CDK4 or CDKN2A/B alterations and IDH-mutant GBM represent a heterogeneous assortment of genomic alterations within the IDH-mutant group with only a few consistent areas of gains and losses (Fig. 5b-c) whereas a large fraction of the CNV in IDH-wildtype tumors arose from consistent amplifications in chromosome 7p (containing EGFR), and deletions in chromosomes 9p and 10 (Fig. 6).
Although the overall CNV changes seem to occur before histologic progression to GBM in cases with other negative prognostic factors and/or clinically demonstrated poor outcomes, there is still uncertainty in the exact connection to elevated levels of CNV and the driving force behind this poor progression. Our data also agrees with the previously demonstrated data that CDK4 and CDKN2A/B alterations are prognostic factors within the IDH-mutant LGGs [44]. While worse prognosis seems to correlate with CDK4 or CDKN2A/B status, our earlier study [36] showed only a fraction of the rapidly progressing tumors had these specific alterations, yet all of them had high overall CNV, indicating that it may be an earlier event or a separate phenomenon altogether. Further analysis of CNV data may help determine if the IDH-mutant LGGs with CDK4 and/or CDKN2A/B alterations are actually early GBMs or simply undersampled tumors, similar to current thinking on many

Table 2 Summary of mutations in genes with known functions related to maintaining DNA and chromosomal stability for each group

| Group | Tumor Type | Mutations in genes with functions related to maintaining overall genome/chromosomal stability |
|-------|------------|------------------------------------------------------------------------------------------|
| 1     | IDH-mut LGG | BRCA2                                                                                     |
| 2     | IDH-mut CDK4/CDKN2A/B LGG | APC, ATM, FANCB, FANCD2, RAD51 (2), TOP1 |
| 3     | IDH-mut GBM  | APC (4), BLM, BRCA2, SMC1 (2)                                                            |
| 4     | IDH-wt LGG   | BLM, FANCB (2), FANC, LIG4                                                              |
| 5     | IDH-wt GBM   | ATR, BRCA2 (2), CLSPN, FANC1 (2), FANCM (2), PRKDC, REV3                              |
The present study reinforces our previous findings [36, 37] demonstrating that elevated CNV is associated with poor outcome in grade II and III IDH-mutant astrocytomas, and presents this as a potential prognostic factor. We demonstrate for the first time that higher CNV is associated with previously established prognostic factors within the IDH-mutant LGG subgroup, such as CDK4 amplification and CDKN2A/B deletion. This study is also the first to demonstrate a significant quantitative difference in mutations of genes related to chromosomal stability in groups with higher CNV and worse clinical outcomes (Fig. 8b).

It is important to note that while many of the genetic and epigenetic methods used to generate these data are currently only used for research purposes, recent proof-of-concept studies have demonstrated that specific and large-scale genetic and epigenetic alterations can be identified rapidly and relatively inexpensively [12, 18], including overall methylation patterns indicative of IDH1/2 status, methylation of key gene promoters, CNV, mutations, and gains and losses of key genes and chromosomal regions. These studies have demonstrated that with newer techniques these molecular factors can be identified in approximately the time that it takes to make a histologic diagnosis. It is therefore conceivable that CNV and other molecular factors identified in this report could soon be used clinically at the time of initial diagnosis to help guide prognosis and treatment strategies.

Conclusions
Our results support our previous findings that IDH-mutant lower-grade astrocytomas with higher total CNV are associated with poor clinical outcome and behave more consistently with IDH-mutant GBM than other IDH-mutant LGGs with low CNV, and suggest that CNV could be a viable prognostic factor in these tumors alongside IDH1/2 mutations, CDK4 amplifications, and CDKN2A/B deletions. We demonstrated that high CNV occurs in IDH1/2-wildtype astrocytomas and glioblastomas which also have poor prognoses, although the reason underlying elevated CNV may be different in IDH-mutant and IDH-wildtype tumors. We also provide a possible mechanism for the overall CNV differences in these astrocytoma subgroups, as the CNV levels seem to correlate with numbers of mutations in genes with roles in maintaining genomic stability. These results suggest that high overall CNV negate the beneficial effects of IDH1/2 mutation, and could potentially be used as a prognostic marker in IDH-mutant astrocytomas in the future.

IDH-wildtype LGGs [3, 42]. While it is reasonable to argue that our cohort of IDH-mutant LGGs without CDK4 or CDKN2A/B alterations show low CNV because they selectively exclude tumors with specific known amplifications/deletions to enrich the other cohorts, if this were to hold true, the clinical outcome would likely also follow the same pattern and would show worse outcome within the other groups containing CDK4 amplification or CDKN2A/B deletion. CDK4 and CDKN2A/B did not show a prognostic difference in IDH-mutant GBMs or IDH-wildtype LGGs or GBMs, and the overall CNV was not different between these two groups (Fig. 2a-c), so the effect of both of these alterations seems limited to IDH-mutant LGG cases. CDK4 amplification and CDKN2A/B deletion also appear to be mutually exclusive, with only two total cases (2.3%) having both molecular alterations (Additional file 4: Figure S4 and Additional file 5: Figure S5).

An additional finding in these tumor groups is the trend toward more frequent mutations in genes associated with overall chromosomal stability in groups with worse clinical outcomes (groups 2–5) compared to the group with relatively favorable outcomes (group 1) (Fig. 8b, Table 2). This correlates positively with the trends toward increased CNV levels and number of cases with chromothripsis and inversely with the progression-free and overall survival in these groups (Table 1). The number of mutations in genes with chromosomal stability functions and cases with chromothripsis are somewhat lower in the IDH-wildtype cohorts compared to groups 2 and 3 in the IDH-mutant cohorts, despite having statistically identical CNV levels (Fig. 8). This difference may be explained by the fact that a large portion of the CNV in these IDH-wildtype groups is more homogeneously associated with specific chromosomal regions (7, 9p, 10) instead of more diffusely distributed as seen in the IDH-mutant groups with high CNV and poor outcome (Figs. 5 and 6).

This process also provides a potential mechanistic explanation for the widespread genomic alterations and the worse prognosis associated with this increase in CNV in at least a subset of cases. Inactivating mutations in genes associated with maintenance of genetic and chromosomal integrity, and the resulting increase in CNV, allows for rapid and widespread changes to the genome, including chromothripsis, and has the potential to cause more frequent gains of oncogenes and loss of tumor suppressor genes and drive tumor formation and progression towards malignancy [11, 19, 20, 41, 46]. This may also suggest a different molecular mechanism underlying total CNV levels in IDH-mutant and IDH-wildtype groups. At this point, however, we can only state that these factors are all correlated with poor clinical outcome, but no causative links can definitively be made.
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Availability of data and materials
The full dataset used in this study is freely available at www.cbioportal.org and https://www.cancer.gov/about-nci/organization/ccg/research/structural-genomics/tcga.

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The authors declare that they have no competing interests.

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