Distinct Genomic Aberrations between Low-Grade and High-Grade Gliomas of Chinese Patients

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Abstract

Background: Glioma is a type of tumor that develops in the central nerve system, mainly the brain. Alterations of genomic sequence and sequence segments (such as copy number variations or CNV and copy neutral loss of heterozygosities or cnLOH) are thought to be a major determinant of the tumor grade.

Methods: We mapped genomic variations between low-grade and high-grade gliomas (LGG and HGG) in Chinese population based on Illumina’s Beadchip and validated the results using real-time qPCR.

Results: At the cytoband level, we discovered: (1) unique losses in LGG on 5q, 8p and 11q, and in HGG on 6q, 11p, 13q and 19q; (2) unique gains in the LGG on 1p and in HGG at 5p, 7p, 7q and 20q; and (3) cnLOH in HGG only on 3q, 8q, 10p, 14q, 15q, 17p, 17q, 18q and 21q. Subsequently, we confirmed well-characterized oncogenes among tumor-related loci (such as EGFR and KIT) and detected novel genes that gained chromosome sequences (such as AASS, HYAL4, NDUFAS and SPAM1) in both LGG and HGG. In addition, we found gains, losses, and cnLOH in several genes, including VN1R2, VN1RA, and ZNF677, in multiple samples. Mapping grade-associated pathways and their related gene ontology (GO) terms, we classified LGG-associated functions as “arachidonic acid metabolism”, “DNA binding” and “regulation of DNA-dependent transcription” and the HGG-associated as “neuroactive ligand-receptor interaction”, “neuronal cell body” and “defense response to bacterium”.

Conclusion: LGG and HGG appear to have different molecular signatures in genomic variations and our results provide invaluable information for the diagnosis and treatment of gliomas in patients with variable duration or diverse tumor differentiation.

Introduction

Glioma is a major tumor type that derives from glial cells of the central nerve system, including of spine and the brain. Gliomas are classified into four grades, from I to IV, with increasing exacerbation according to histology [1]. Alternatively, they are categorized into ependymomas, astrocytomas, oligodendrogliomas, and mixed gliomas, according to the cell types that are anatomically defined from different brain regions based on the brain topology [2]. Glioma is considered as low prevalence but an invaluable information for the diagnosis and treatment of gliomas in patients with variable duration or diverse tumor differentiation.

therapies for the tumors, such as surgery, radiotherapy, chemotherapy, and oral medication, are often individually or selectively cooperated to remit or to cure the symptoms according to patient’s condition and tumor grade [5,6]. Although the degree of copy number variations (CNVs) among healthy human populations do vary, abnormal increase of CNVs and cnLOHs are thought to be at least one of the important causative factors for gliomas and other cancers [7,8,9]. Large-scale genomic aberration and gene expression studies on gliomas have lead to identifications of genes, which are involved in numerous cellular functions and metabolic pathways (PCHS9, CXCL2, MYC, PDGFR, PARK2, DM1BT1, TOP2A, PTEN, ARF, TP53, P16, CDKN2B, RB1, EGFR, and NF1 [9,10,11,12,13,14,15,16,17]), as well as those related to neural development, cell signaling (IAS/RAF, RTK, MAPK, PI3K, and ROCK), and tumor suppression (p53 and RB)
These genes serve as biomarkers useful for tumor classification, prognosis, and typing, in-depth study on special tumor types become feasible, especially when two novel parameters—physical (Log R ratio) and genetic (B allele frequency) alterations/variability—are used to increase sensitivity and accuracy [45]. We report the first study in which we compared chromosomal aberrations of low-grade and high-grade gliomas (LGG and HGG) in Chinese populations to unveil differences of tumor-related sequence variations, including chromosomes and cytobands, and individual genes in the context of pathways and function categories.

Materials and Methods

Ethics Statement

This study was approved by the ethics committee at First Hospital of Jilin University, and all patients who agreed to this study signed their informed consents. In particular, some of the written informed consents were also obtained from the next of kin, caretakers, or guardians on behalf of the minors/children participants involved in this study.

Sample Preparation and Genotyping

18 gliomas, including nine of each for both low-grade (grade II) and high-grade (grade III and 4 grade IV) tumors (as LGG and HGG), as well as 18 paired blood samples were collected at First Hospital of Jilin University (Table 1). In general, patients with LGG (average age of 41) and HGG (average age of 47) have no prior history of chemotherapy or radiation treatment, and the tumors and matched peripheral blood samples were obtained at the time of tumor resection. The blood samples were immediately snap-frozen in liquid nitrogen and stored at −80°C before DNA extraction. Portions of the solid tumors were treated in the same way as the blood samples and others were paraffin-embedded for histopathology. The genomic DNA was isolated by using QIAamp DNA Mini Kit (QIAGEN Inc). DNA quality and purity were determined based on agarose gel electrophoresis and spectrophotometry. Four additional gliomas for RNA RT-qPCR validation were also collected at the same hospital and treated according to the same protocol as used for the other samples (Table 1). Genotyping was carried out by using Illumina Sentrix Human Genomic Aberration Patterns in Gliomas

| ID  | Gender | Age | Type of glioma | Grade | Type of experiment |
|-----|--------|-----|----------------|-------|-------------------|
| S1  | Male   | 40  | astrocytoma    | II    | Chip              |
| S2  | Female | 13  | diffuse astrocytoma | II    | Chip              |
| S3  | Male   | 37  | diffuse astrocytoma | II    | Chip              |
| S4  | Male   | 44  | diffuse astrocytoma | II    | Chip              |
| S5  | Female | 33  | diffuse astrocytoma | II    | Chip              |
| S6  | Male   | 48  | diffuse astrocytoma | II    | Chip              |
| S7  | Female | 32  | medulloblastoma | II    | Chip              |
| S8  | Female | 63  | oligodendroglioma | II    | Chip              |
| S9  | Male   | 55  | oligodendroglioma | II    | Chip              |
| S10 | Male   | 37  | anaplasia oligodendroglioma | III | Chip              |
| S11 | Female | 55  | anaplastic astrocytoma | III | Chip              |
| S12 | Male   | 57  | anaplastic astrocytoma | III | Chip              |
| S13 | Female | 43  | anaplastic astrocytoma | III | Chip              |
| S14 | Male   | 39  | anaplastic astrocytoma | III | Chip              |
| S15 | Female | 55  | astrocytoma      | III    | Chip              |
| S16 | Female | 41  | glioablastoma    | IV     | Chip              |
| S17 | Female | 55  | glioablastoma    | IV     | Chip              |
| S18 | Male   | 44  | mixed oligodendroglioma | IV | Chip              |
| S19 | Male   | 39  | anaplasia oligodendroglioma | II | RT-qPCR           |
| S20 | Male   | 7   | medulloblastoma  | III    | RT-qPCR           |
| S21 | Male   | 11  | glioablastoma    | IV     | RT-qPCR           |
| S22 | Female | 59  | glioablastoma    | IV     | RT-qPCR           |

Note: RT-qPCR: real-time quantitative PCR.
doi:10.1371/journal.pone.0057168.t001

Table 2. Primers and results of real-time qPCRs.

| Gene  | Primer                      | Product size (bp) | Fold change |
|-------|-----------------------------|-------------------|-------------|
| AASS  | F: AGCGCTACATAAGTCGTT        | 197               | 1.86        |
|       | R: GTCCACATATTGCCACAGGTTT    |                   |             |
| CYP2J2 | F: AAAGAAGGCTCTTATCACC      | 187               | 30.53       |
|       | R: GAATGCCGTTCTTCAAAGCTCT    |                   |             |
| CYP4A11 | F: CCTGGACAGATGGCTCACC      | 107               | 111.92      |
|       | R: CAGGGGAGTGGACGGGAGT       |                   |             |
| PLA2G2A | F: CATGATTTTGGGCTACTGC     | 108               | 6.57        |
|       | R: CAGCGCGTAAAGCCTAACTG      |                   |             |
| PLA2G5 | F: ACATCCGACACACGTCTAC      | 170               | 24.23       |
|       | R: GAGGGTGTTGGGAAATGTT       |                   |             |
| PTEN  | F: CAGAAAGACTTGAGGCGGTAT    | 124               | 52.53       |
|       | R: TTGGGGTGCATATAATG         |                   |             |
| RB1   | F: TCTCGAAGGCCTCTAAGT       | 176               | 6.38        |
|       | R: TCTCAGAAATTCGGATG         |                   |             |

Note: These primers were designed by using Oligo6. Fold changes were computed by comparing between HGG and LGG.

doi:10.1371/journal.pone.0057168.t002

Overview of samples used in this study.

| Sample Preparation and Genotyping | Sample Preparation and Genotyping |
|----------------------------------|----------------------------------|
| Ethnics Statement                | Materials and Methods            |
| Ethics Statement                 | Materials and Methods            |
| Sample Preparation and Genotyping| Sample Preparation and Genotyping|
| Experiment                        | Experiment                        |
Table 3. Comparison of sample numbers of genomic aberration in gliomas at the cytoband level.

| Homozygous deletions | L 8p11.23 | 2 |
|----------------------|-----------|---|
|                      | H 13q12.11 | 2 |
| Hemizygous deletions | L 5q14.3 | 2 |
|                      | 11q11 | 2 |
|                      | H 6q12 | 2 |
|                      | 6q13 | 2 |
|                      | 6q14.1 | 2 |
|                      | 6q14.2 | 2 |
|                      | 6q14.3 | 2 |
|                      | 6q16.3 | 2 |
|                      | H 6q21 | 2 |
|                      | 6q22.1 | 2 |
|                      | 6q22.31 | 2 |
|                      | H 6q22.32 | 2 |
|                      | 6q22.33 | 2 |
|                      | 6q23.1 | 2 |
|                      | H 6q23.2 | 2 |
|                      | 6q23.3 | 2 |
|                      | 6q24.1 | 2 |
|                      | H 6q24.2 | 2 |
|                      | 1p15.4 | 2 |
|                      | 13q12.2 | 2 |
|                      | H 13q12.3 | 2 |
|                      | 13q13.1 | 2 |
|                      | 13q13.2 | 2 |
|                      | H 13q14.12 | 2 |
|                      | 13q14.13 | 2 |
|                      | 13q14.2 | 2 |
|                      | H 13q14.3 | 2 |
|                      | 13q21.1 | 2 |
|                      | 13q21.2 | 2 |
|                      | H 13q21.31 | 2 |
|                      | 19q12 | 2 |
|                      | 19q13.11 | 2 |
|                      | H 19q13.12 | 2 |
| cnLOHs               | L 2p12 | 2 |
|                      | 6p21.32 | 2 |
|                      | 6p21.33 | 2 |
|                      | L 6p22.1 | 2 |
|                      | 6q15 | 2 |
|                      | 9p24.1 | 2 |
|                      | L 22q12.3 | 2 |
|                      | H 2p23.2 | 2 |
|                      | 2p25.1 | 3 |
|                      | 2p25.3 | 2 |
|                      | H 3q26.1 | 2 |
|                      | 6p21.32 | 3 |
|                      | 6p21.33 | 3 |
|                      | H 6q25.1 | 2 |
|                      | 8q22.3 | 2 |
|                      | 8q24.3 | 3 |
|                      | H 9p24.1 | 2 |
|                      | 9p24.2 | 2 |
|                      | 10p13 | 2 |
|                      | H 10p15.1 | 2 |
|                      | 14q32.13 | 2 |
|                      | 15q26.1 | 2 |
|                      | H 17p13.3 | 2 |
|                      | 17q22 | 2 |
|                      | 18q22.1 | 2 |
|                      | H 18q22.3 | 2 |
|                      | 18q23 | 2 |
|                      | 21q22.3 | 2 |
|                      | H 22q13.31 | 3 |
| Duplications         | L 1p13.3 | 4 |
|                      | 1p21.1 | 4 |
|                      | 1p21.3 | 4 |
|                      | L 1p22.2 | 4 |
|                      | 1p22.3 | 4 |
|                      | 1p31.1 | 4 |
|                      | L 1p31.3 | 4 |
|                      | 1p32.1 | 4 |
|                      | 1p32.2 | 4 |
|                      | L 1p32.3 | 4 |
|                      | 1p33 | 4 |
|                      | 1p34.4 | 4 |
|                      | L 1p35.1 | 4 |
|                      | 1p35.2 | 4 |
|                      | 1p35.3 | 4 |
|                      | L 1p36.11 | 4 |
|                      | 1p36.12 | 4 |
|                      | 1p36.13 | 4 |
|                      | L 1p36.31 | 4 |
|                      | H 5p15.33 | 4 |
|                      | 7q31.31 | 4 |
|                      | 7q31.32 | 4 |
|                      | H 7q31.33 | 4 |
|                      | 7q33 | 4 |
|                      | 7q34 | 4 |
|                      | H 7q35 | 4 |
|                      | 7q36.1 | 4 |
|                      | 20q11.1 | 4 |
| Amplifications       | H 7p11.2 | 2 |

Note: L and H stand for LGG and HGG, respectively.
doi:10.1371/journal.pone.0057168.t003

CNV370-Quad v3 BeadChip according to the manufacturer’s instruction.

Data Analysis and Functional Annotation

Illumina’s KaryoStudio (V1.0.3), together with the cnPartition algorithm, was adopted to identify CNV regions. Illumina’s GenomeStudio software (V2009.1) with LOHscore plug-in was used to discover copy neutral loss of heterozygosity (cnLOH) regions. The samples were divided into LGG and HGG, and the analysis was limited to autosomal regions only due to the experiment design. The data used in this study were submitted to GEO with an accession of GSE34888.

GeneCoDis3 was used to relate genes to the corresponding Gene Ontology (GO) terms with cut-off = 0.05 [46], and WebGestalt V2 was used to map genes onto significant KEGG pathways with cut-off = 0.1 [47]. OMIM (Online Mendelian Inheritance in Man) was used to find relationship between genes and diseases [49].

Real-time qPCR-based Validation Experiment

Four RNA samples, reverse-transcribed (Table 1), were subjected to real-time qPCR in a final reaction volume of 10 µl, which contains: 1 µl of 10X buffer, 0.25 µl of dNTP, 2 µl of gene-specific forward and reverse primer mix (Table 2), 1 µl cDNA (100 ng/µl), 0.1 µl of Taq, 0.5 µl of fluorochrome (TIANGEN BIO TECH) and 5.15 µl of ddH2O.

Results

Case and Control

Using 18 solid tumors from patients (9 as LGG and 9 as HGG) as the case sample and the patients’ DNA isolated from their peripheral blood as the control, we compared chromosome aberrations between the case and control based on the Illumina’s BeadChip platform that defines both copy number variation (CNVs) and copy neutral loss of heterozygosity (cnLOH). This technology allows us to find sequence differences between tumor and blood samples at cytogenetic and molecular levels, which include both gains and losses. We only discovered sequence amplifications in the tumor not in the corresponding periphery blood. In other three aberration categories of CNVs, there are always more variations in the tumor than in the control, and the significance of variations between the paired samples can be evaluated statistically (P-value < 0.05, paired t-test) when the total length influenced by CNVs in each sample was considered.

CNVs among Autosomal Arms

After the survey of different CNVs in 44 autosomal arms in LGG and HGG, we removed those shared by tumors and their controls to identify tumor-specific variations. We made a few interesting observations. First, 13q harbors a major high-frequency genomic aberration class. For instance, there are 3, 3, 3, 2, and 2 samples found to have homozygous deletion, hemizygous deletion, cnLOHs, duplication and amplification, respectively. Second, we found that 22, 29, 38, 38, and 6 chromosomal arms have variations in the five categories, respectively. Obviously, cnLOH is one of the most frequent categories, and our results agreed with a previous report [43]. In addition, the skewed distribution of sequence amplification showed that there were more copy number gains found on only a few chromosomal arms. Third, we paid more attention to CNVs between LGG and HGG and found several homozygous deletions of 13q in HGG but not in LGG. There were also cnLOHs in three HGG, such as those on 15q and 17q, but none was found in LGG. In addition, four HGG had duplications on 3q but none was found in LGG.

Comparison of LGG and HGG at the Cytoband Level

To pinpoint the position of variations on chromosomes, we investigated CNV and cnLOH in LGG and HGG. To reduce false positives, we only referred to those that occurred in at least two tumor samples (four samples for “duplications” category) after removing what appeared in the controls, i.e., corresponding blood samples. We only discovered sequence differences between tumor and blood samples at cytogenetic and molecular levels, which include both gains and losses.

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duplication events in LGG than in HGG. Taken together, we conjectured that losses and gains occur more often in HGG and LGG, respectively. Furthermore, we found that gains usually occurred on 7q and 1p in HGG and LGG, respectively. Decreasing copy number is usually discovered on 6q, 13q and 19q in HGG. We also noticed that there were overlaps between the two tumor grades in the cnLOH category, especially the fact that cnLOHs were spread out more broadly in cross-chromosome cytobanding than the rest of copy number variation categories.

Genes Found to Associate with Gliomas

To identify specific genes associated with gliomas, we pooled all genomic aberrations occurred in at least six tumor samples. After filtered our data based on known variations found in the controls and Database of Genomic Variants (hg18.v8) (http://projects.tcag.ca/variation/), we had 24 genes and the related information was summarized (chromosome location, aberration category, tumor grading) (Table 4). These genes are all clustered on 1p, 7q, and 19q. Among them, 17 genes are only gains, and three of other genes, VN1R2, VN1R4 and ZNF677, have all three types of genomic aberrations—gain, loss and cnLOH. Referencing to the annotation of the OMIM Morbid Map (http://www.ncbi.nlm.nih.gov/omim), we found that AASS, TAS2R16 and TSPAN12 are previously identified to be disease-related and associated with “hyperlysinemia”, “alcohol dependence” and “exudative vitreoretinopathy”, respectively.

Pathway Enrichment and Functional Annotation of the Associated Genes

To decipher functional relevance of genes and gene classification related to gliomas, we established datasets based on the following two criteria: (i) elimination of genes shared by both the case and the control and (ii) removal of genes shared by less than four samples. Altogether, we collected 442 LGG and 111 HGG associated genes. After mapping the two groups of genes onto the KEGG pathways, we obtained 12 pathways for LGG, which belong to “lipid metabolism” (in the number of pathways: 6), “neurodegenerative diseases” (1), “endocrine system” (1), “nervous system” (1), “circulatory system” (1), “signal transduction” (1), and “immune system” (1) (Table 5). These genes were categorized in 10 pathways correlated to HGG, among which two were classified as “lipid metabolism” and others were distributed in diverse classifications such as “signaling molecules and interaction”, “signal transduction”, “endocrine system”, “carbohydrate metabolism”, “amino acid metabolism”, “glycan biosynthesis and metabolism”, and “infectious diseases”. The most four significant pathways identified in LGG are “arachidonic acid metabolism”, “linoleic acid metabolism”, “alpha-Linolenic acid metabolism”, and “ether lipid metabolism”. In HGG, the most four enriched terms are “metabolic pathways”, “neuroactive ligand-receptor interaction”, “calcium signaling pathway”, and “melanogenesis”. We found 12 and 4 enriched GO terms associated with LGG and HGG, respectively (Table 6). In LGG, the top two GO terms were “neuronal cell body” and “defense response to bacterium”. To

| Gene       | Description                                      | Location | Total | Gain | Loss | cnLOH | LGG | HGG |
|------------|--------------------------------------------------|----------|-------|------|------|-------|-----|-----|
| NKAIN1     | Na+/K+-transporting ATPase interacting 1         | 1p       | 6     | 5    | 1    | 4     | 2   |     |
| PTPRU      | Protein tyrosine phosphatase, receptor type, U   | 1p       | 6     | 5    | 1    | 4     | 2   |     |
| SLC4A3     | Solute carrier family 44, member 3               | 1p       | 6     | 5    | 1    | 4     | 2   |     |
| AASS       | Aminoadipate-semialdehyde synthase               | 7q       | 6     | 6    |      |       |     | 4   |
| ASB15      | Ankyrin repeat and SOCS box-containing 15        | 7q       | 6     | 6    |      |       | 2   | 4   |
| C7orf58    | chromosome 7 open reading frame 58              | 7q       | 6     | 6    |      |       | 2   | 4   |
| FEZF1      | FEZ family zinc finger 1                        | 7q       | 6     | 6    |      |       | 2   | 4   |
| GPR37      | G protein-coupled receptor 37 (endothelin receptor type B-like) | 7q | 6 | 6 | 2 | 4 |
| HYAL4      | Hyaluronoglucosaminidase 4                      | 7q       | 6     | 6    |      |       | 2   | 4   |
| ING3       | Inhibitor of growth family, member 3            | 7q       | 6     | 6    |      |       | 2   | 4   |
| IQUB       | IQ motif and ubiquitin domain containing         | 7q       | 6     | 6    |      |       | 2   | 4   |
| LMOD2      | Leiomodin 2 (cardiac)                           | 7q       | 6     | 6    |      |       | 2   | 4   |
| NDUFA5     | NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 5, 13 kDa | 7q | 6 | 6 | 2 | 4 |
| POT1       | POT1 protection of telomeres 1 homolog (S. pombe) | 7q | 6 | 6 | 2 | 4 |
| RNF133     | Ring finger protein 133                         | 7q       | 6     | 6    |      |       | 2   | 4   |
| RNF148     | Ring finger protein 148                         | 7q       | 6     | 6    |      |       | 2   | 4   |
| SPAM1      | Sperm adhesion molecule 1                       | 7q       | 6     | 6    |      |       | 2   | 4   |
| TAS2R16    | Taste receptor, type 2, member 16               | 7q       | 6     | 6    |      |       | 2   | 4   |
| TSPAN12    | Tetraspanin 12                                  | 7q       | 6     | 6    |      |       | 2   | 4   |
| WASL       | Wiskott-Aldrich syndrome-like                   | 7q       | 6     | 6    |      |       | 2   | 4   |
| VN1R2      | Vomeronasal 1 receptor 2                       | 19q      | 6     | 4    | 1    | 1     | 5   | 1   |
| VN1R4      | Vomeronasal 1 receptor 4                       | 19q      | 6     | 4    | 1    | 1     | 5   | 1   |
| ZNF507     | Zinc finger protein 507                        | 19q      | 6     | 3    | 3    |       | 4   | 2   |
| ZNF677     | Zinc finger protein 677                        | 19q      | 6     | 4    | 1    | 1     | 5   | 1   |

doi:10.1371/journal.pone.0057168.t004
our current knowledge, tumors often alter cellular processes, such as proliferation, growth, programmed death, differentiation, division, mutation-induced DNA damage, and repair [1]. All these newly discovered distinct function categories may introduce features of primary (low-grade) or malignant (high-grade) tumors.

Correlating Copy Number Variation with Gene Expression

We used real-time qPCR to validate the expression of seven identified genes: AASS, CYP2J2, CYP4A11, PLA2G2A, PLA2G5, PTEN, and RB1. First, all genes showed increased expression when compared in the two tumor grades (HGG vs. LGG) (Table 2). CYP2J2, CYP4A11, PLA2G5 and PTEN exhibited even over 10 fold changes. We did not compare the gene expressions between the tumors and the controls, their corresponding blood cells, due to the tissue-specific nature of the gene expression. Of the seven genes, we noticed RB1 gains in HGG and losses in both grades. Other different aberrations include (1) CYP2J2, CYP4A11, PLA2G5 and PTEN exhibited even over 10 fold changes. We did not compare the gene expressions between the tumors and the controls, their corresponding blood cells, due to the tissue-specific nature of the gene expression. Of the seven genes, we noticed RB1 gains in HGG and losses in both grades. Other different aberrations include (1) CYP2J2, CYP4A11, PLA2G5 and PTEN gains in HGG, (2) AASS gain in both, and (3) PTEN gains in HGG only. The correlation between expression and copy number is complex, and has been demonstrated to be positive under most conditions with several exceptions of negative correlations [12]. There are a few interpretations for the complicated relations between gene (or segment) copy number and gene expression. First, the higher gene dosage leads to an increased transcript production and a positive correlation. Second, negative correlation has been proposed that some possible compensation or time-series feedback effects may act on the progression or cell response of tumors [12]. Last, a handful of CNVs can destroy regulatory regions to some extents and inhibit the expression of relevant genes. In any case, we have found significant differential gene expressions in selected genomic structures and genes between LGG and HGG.

Discussion

In accordance with Knudson’s two-hit hypothesis on tumor formation [49], tumorigenesis occurs concurrently with activation of oncogenes and inactivation of tumor suppressor genes (TSGs). In particular, tumor formation is a process where the potential for malignancy increases with mutation accumulation [50]. Our results support the concept that genomes in HGG tend to be deleted more intensively than those in LGG at both cytoband and molecular levels. In other words, there are gains of genes or partial sequences in the primary stage of the tumors and subsequently losses of tumor suppressor genes or TSGs appear to escape from normal cellular controls. For instance, cnLOHs in HGG happened in twelve chromosomes (2, 3, 6, 8, 9, 10, 14, 15, 17, 18, 21, and 22). In contrast, cnLOHs in LGG occurred only in

| Table 5. Pathway analysis of genes involved in genomic aberration in LGG (A) and HGG (B). |

| (A) LGG | Pathway name | Observed number | Expected number | R     | FDR   |
|---------|--------------|-----------------|-----------------|-------|-------|
| Arachidonic acid metabolism | 9 | 1.35 | 6.69 | 1.00E−04 |
| Linoleic acid metabolism | 7 | 0.67 | 10.41 | 1.00E−04 |
| alpha-Linolenic acid metabolism | 6 | 0.43 | 13.87 | 1.00E−04 |
| Ether lipid metabolism | 7 | 0.82 | 8.57 | 2.00E−04 |
| Glycerophospholipid metabolism | 8 | 1.61 | 4.97 | 2.10E−03 |
| Prion diseases | 6 | 0.84 | 7.14 | 2.10E−03 |
| GnRH signaling pathway | 9 | 2.38 | 3.78 | 5.50E−03 |
| Long-term depression | 7 | 1.63 | 4.28 | 9.60E−03 |
| Vascular smooth muscle contraction | 9 | 2.71 | 3.32 | 1.14E−02 |
| VEGF signaling pathway | 7 | 1.78 | 3.94 | 1.28E−02 |
| Fc epsilon III signaling pathway | 7 | 1.85 | 3.78 | 1.45E−02 |
| Fatty acid metabolism | 4 | 1.01 | 3.96 | 9.55E−02 |

| (B) HGG | Pathway name | Observed number | Expected number | R     | FDR   |
|---------|--------------|-----------------|-----------------|-------|-------|
| Metabolic pathways | 14 | 6.21 | 2.25 | 6.00E−02 |
| Neuroactive ligand-receptor interaction | 5 | 1.48 | 3.37 | 6.00E−02 |
| Calcium signaling pathway | 4 | 1.02 | 3.92 | 6.00E−02 |
| Melanogenesis | 3 | 0.58 | 5.14 | 6.00E−02 |
| Fructose and mannose metabolism | 2 | 0.2 | 10.08 | 6.00E−02 |
| Lysine degradation | 2 | 0.25 | 7.97 | 6.00E−02 |
| Androgen and estrogen metabolism | 2 | 0.26 | 7.62 | 6.00E−02 |
| Glycerolipid metabolism | 2 | 0.25 | 7.97 | 6.00E−02 |
| Glycine, serine and threonine metabolism | 2 | 0.12 | 16.32 | 6.00E−02 |
| Vibrio cholerae infection | 2 | 0.31 | 6.47 | 7.28E−02 |

Note: R indicates the ratio of enrichment.
doi:10.1371/journal.pone.0057168.t005
four chromosomes (2, 6, 9, and 22) (Table 3). This particular clear observation in cnLOHs has not been found in other variation types, and the result indicates that disappearance of heterozygosity and allelic losses together with gene functions are major contributors to HGG.

We have effectively validated many locations, genes, pathways, and function categories in keeping with the previous studies at different levels. At the chromosome level, among the five variation classes, cnLOH is the major type, which is distributed over all chromosomes. Furthermore, we have validated many variations at the cytoband level (Table 3). For instance, we found duplications on 7q31.31, 7q31.32, 7q31.33, 7q33, 7q34, 7q35 and 7q36.1 in HGG, which is in agreement with gains of 7q reported in both grades [37]. We discovered hemizygous deletions on numerous cytobands (e.g., 6q12, 6q13, 6q14.1, 6q16.3, 6q21, 6q22.1, 6q23.1, 6q24.1, 13q12.2, 13q13.1, 13q14.12, 13q21.1, 19q12, and 19q13.1) in line with losses of 6q, 13q, and 19q in HGG [37,44]. There have been some contrasting results in this study; for example, we only identified duplications on 1p in LGG and hemizygous deletions on 19q in HGG but LOHs on 1p-19q were reported in both grades by another group [41]. We also have some novel findings specific to Chinese populations and observed the complicated and altered roles of traditional tumor-related genes in our study. For example, \( KIT \) gains in 2 LGG and 2 HGG, \( MGMT \) gains in 1 LGG and 3 HGG, \( MDM2 \) gains in 1 LGG and 3 HGG, \( DMBT1 \) gains in 1 LGG and 3 HGG, \( IDH1 \) only gains in one LGG. Thus, TSGs and oncogenes are relative and condition-sensitive concept, i.e., TSGs in one sample may be oncogenes in another. We finally inferred that TSGs not only can be lost but also can be gained, whereas oncogenes appear generally being gained in these tumors.

We did not find any obvious major pathways shared by LGG and HGG but identified 6 and 2 subclasses from the major class “lipid metabolism” shared by the two grades, respectively. In HGG, we noticed some interesting variations, including \( AASS \) (4 gains) in “lysine degradation” and \( CHRM2 \) (4 gains), \( CYSLT2 \) (2 gains and 2 losses), \( GRM8 \) (4 gains), \( HTR2A \) (2 gains and 2 losses), and \( MLNR \) (2 gains and 2 losses) in “neuroactive ligand-receptor interaction”. We further found variations of \( GRB2 \) (2 gains and 2 losses) and \( CYSLT2 \) (1 loss), \( HTR2A \) (1 loss), \( MLNR \) (1 loss) in LGG. Gains in the four LGG are \( CYP2J2 \), \( CYP4A11 \), \( CYP4A22 \), \( PLA2G2A \), \( PLA2G2E \), \( PLA2G2F \), \( PLA2G5 \), and they are involved in “arachidonic acid metabolism”. Looking into well-known oncogenes and TSGs, we identified gains of \( EGFR \) in “de novo glioma pathway” (2 in LGG and 3 in HGG), as well as \( MDM2 \) (2 in LGG and 3 in HGG) and \( PTEN \) (1 in HGG), and losses of \( CDKN2A \) (2 in HGG). In “secondary glioma pathway”, gains of \( PDGFA \) (1 in LGG and 2 in HGG), \( PDGFR \) (2 in LGG and 2 in HGG) and \( CDK4 \) (1 in LGG and 2 in HGG) and \( RB1 \) (2 in HGG), and losses of \( RB1 \) (1 in LGG and 2 in HGG) are also identified.

Our study was designed to uncover molecular differences between LGG and HGG, which are largely based on pathology, morphology, and degree of malignancy, and for which we know that altered genomic regions of HGG are more severe than those of LGG and that patient survival time is shorter in HGG (data not shown). Based on our study, we hope to associate genetics to clinical outcomes in three crucial aspects of fighting the disease, including diagnosis, treatment, and prognosis. First, specific and
dominant genomic variations in cytobands or genes may be used to classify the two tumor grades at molecular level. Second, different genes with DNA aberrations may be further pursued as potential drug targets, especially in the personalized or population-specific cancer treatment for Chinese populations. Third, the disease-associated genes from LGG and HGG may be used for inferring survival time and guidance for disease treat [51,52,53]. In addition, we proposed a theory that losses of genes or alleles may play greater role in the formation and progression of HGG although both gains and losses contribute. This phenomenon is only observed at the cytoband level and relevant to concentrated alternations of homologous or heterogeneous genome composition [54]. Therefore, this is not a decisive but a substantial measure for glioma morbidity. Further large-scale investigations are needed for further validations.

Ultimately, we should be able to build an intricate network that is composed of pathways involved in gliomas both identified in this study and other known in the previous literature as we exemplified in Figure 1. The first characteristic of the network is the difficulty to fully discriminate all three complex modules of the pathways, such as LGG, HGG and others. However, we are able to identify major hubs among the modules, such as MP and MAPK, in the categories of HGG and others, respectively; some may even be obviously LGG associated, such as AAM. In addition, the tissue-specificity of gliomas is blurred by AP and other five vertices including PD, ALS, AD, HUD and PAD, which are common for neurodegenerative diseases and other neuro-pathological conditions. As a final note, the patterns of the three categories may suggest that there are differences in disease-causing mechanisms between Chinese and other populations. It is undoubted that studies on larger samples, coupled with in-depth analyses and validations, are what we should do in the near future.

Figure 1. A network of pathways related to LGG, HGG, and others. The abbreviations for KEGG pathways used in this analysis are listed as follows. AAM: Arachidonic acid metabolism; LAM: Linoleic acid metabolism; ALAM: alpha-Linolenic acid metabolism; ELM: Ether lipid metabolism; GPM: Glycerophospholipid metabolism; PD: Prion diseases; GNRH: GnRH signaling pathway; LTD: Long-term depression; VSMC: Vascular smooth muscle contraction; VEGF: VEGF signaling pathway; FCER: Fc epsilon RI signaling pathway; FAM: Fatty acid metabolism; MP: Metabolic pathways; NLI: Neuroactive ligand-receptor interaction; CSP: Calcium signaling pathway; ME: Melanogenesis; FMM: Fructose and mannose metabolism; LD: Lysine degradation; AEM: Androgen and estrogen metabolism; GM: Glycerolipid metabolism; GD: Glycosaminoglycan degradation; VCI: Vibrio cholerae infection; AD: Alzheimer’s disease; PD: Parkinson’s disease; ALS: Amyotrophic lateral sclerosis; HUD: Huntington’s disease; PI3K: PI3K-Akt signaling pathway; HIF-1: HIF-1 signaling pathway; MAPK: MAPK signaling pathway; CC: Cell cycle; PI3: p53 signaling pathway; AP: Apoptosis; GL: Glioma; ERBB: ErbB signaling pathway; WNT: Wnt signaling pathway; and OP: Oxidative phosphorylation.

doi:10.1371/journal.pone.0057168.g001
Author Contributions
Conceived and designed the experiments: SLF FZ JY DMW. Performed the experiments: YBL LW FZ. Analyzed the data: DPW LW. Contributed reagents/materials/analysis tools: YBL JHY DHD YC PG FZ. Wrote the paper: DPW JY.

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