Molecular determinants of neurocognitive deficits in glioma: based on 2021 WHO classification

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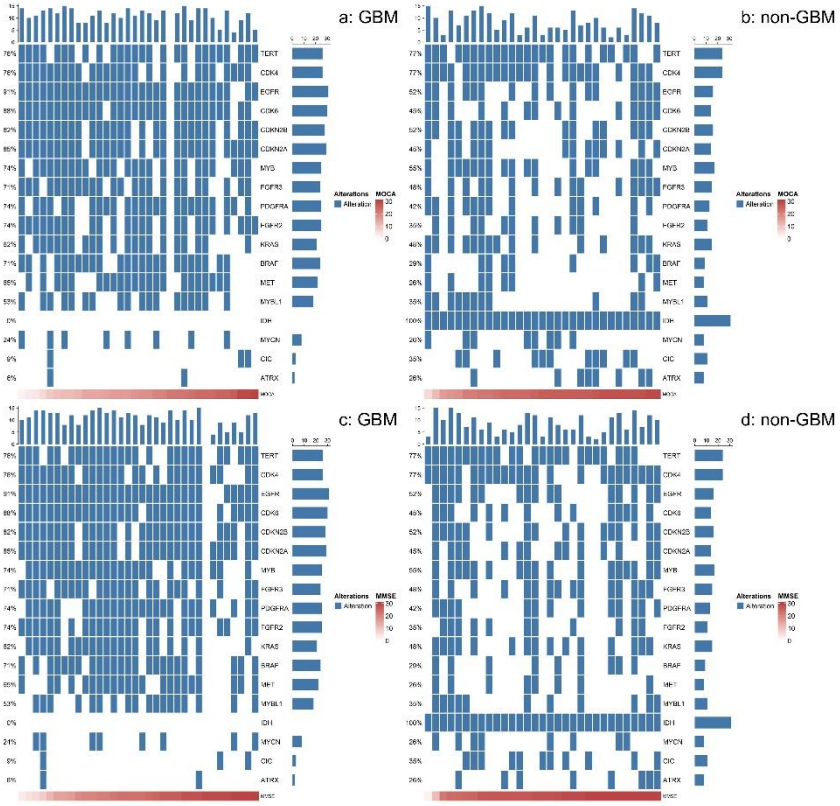

Supplementary figure 1 Waterfall plot of genetic alteration. **a** shown as MOCA score for GBM patients. **b** shown as MOCA score for non-GBM patients. **c** shown as MMSE score for GBM patients. **d** shown as MMSE score for non-GBM patients

Supplementary document 1

Methods for DNA sequencing

Step1. DNA extraction and Library preparation

A total of 65 formalin-fixed paraffin-embedded (FFPE) tumor tissue was obtained from Peking Union Medical College Hospital (PUMCH). DNA extraction was performed with AllPrep DNA/RNA FFPE Tissue Kit (QIAGEN 56404). Nucleotide concentrations were determined by Qubit 4.0 Fluorometer (Thermo Fisher Scientific) using the dsDNA HS Assay Kit (Invitrogen, Q32854). Nucleotide purity was determined by Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific). NGS libraries were generated with the NEBNext Ultra II DNA Library Prep Kit for Illumina (New England Biolabs) and the The KAPA Target Enrichment Probes package (Roche) was used to perform enrichment of targeted regions. The concentrations of the sub and final libraries were determined using Qubit 4.0. The quality control results showed that the fragment size of the library was about 350 bp and the total amount of libraries was >500 ng.

Step2. Next generation sequencing and analysis of NGS data

Sequencing runs were either performed on the NovaSeq6000 with loading concentration of 240 pm. Sentieon was used to perform sequence comparison. Clean Reads is about 5G，mapping rate>99%, average sequencing depth >500X and target capture rate is 75%. Base Quality Score Recalibration (BQSR) was used to corrected base quality. Single nucleotide polymorphisms(SNP) also insertions and deletions (indels) were detected with TNscope. Copy number variations(CNVs) were identified with Cnvkit. The screening criteria for CNV is as follow:

[1] Deletion: indicates a deletion of the segment, which can be interpreted as a copy number decrease; judgment condition: the ratio of copy number <= 1.5 bin >= 0.3.

[2] Amplification: indicates that the region is amplified, which can be interpreted as copy number increase; judgment condition: the ratio of copy number >= 2.5 bin >= 0.3.

[3] Amplification/deletion: indicates that the region is amplifying and deletion; judgment condition: the ratio of copy number >= 2.5 bin >= 0.3 and the ratio of copy number <= 1.5 bin >= 0.3.

[4] Mix: indicates that a copy number change has occurred in the segment, but it is not sure whether it is an amplification or a deletion; judgment condition: (ratio of copy number <= 1.5 bin + ratio of copy number >= 2.5 bin) >= 0.3

The cut off of variant allele frequency (VAF) for hotspot mutation is 5% and 10% for non-hotspot mutation. The variation should be in exon region and not be synonymous mutation. Also, the variation should not be contained in the non-oncology population database of Genome Aggregation Database(GnomAD), East Asian Healthy People Database, and the Southern Chinese Han Population Database.