**Supplementary material**

**Diencephalic Pediatric Low-grade Glioma Harboring the BRAF V600E Mutation Presenting with Various Morphologies in Sequential Biopsy Specimens**

**Brain Tumor Pathology**

Yukitomo Ishi, Kanako C. Hatanaka, Shigeru Yamaguchi, Hiromi Fujita, Hiroaki Motegi, Hiroyuki Kobayashi, Shunsuke Terasaka, Kiyohiro Houkin

Corresponding Author:

Shigeru Yamaguchi, MD

Department of Neurosurgery, Hokkaido University Graduate School of Medicine

E-mail: [yama-shu@med.hokudai.ac.jp](mailto:yama-shu@med.hokudai.ac.jp)

Procedure for genetic analysis

Prior to genetic analysis, we sought appropriate approval from our local institutional review board and obtained written informed consent from the patient’s parents. DNA/RNA was extracted from the tumor using an AllPrep DNA/RNA Mini Kit (Qiagen, Tokyo, Japan), and first-strand cDNA was synthesized using a PrimeScript® II 1st strand cDNA Synthesis Kit (Takara, Tokyo, Japan) in accordance with the manufacturer’s recommendations. Two hotspots within the *TERT* promoter (C228T, C250T), along with mutation hotspots at codon 132 of *IDH1*, codon 172 of *IDH2*, codon 27 and 36 of *H3F3A,* and codon 600 of *BRAF* were screened using Sanger sequencing. Templates for Sanger sequencing were prepared by ampli­fying genomic DNA using Premix Taq (Takara, Tokyo, Japan). The oligonucleotide primers used for amplification are presented in the following Table. References of primers previously reported are described in the Reference list. Cycle sequencing was carried out using the BigDye Ter­minator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) using the forward and reverse PCR primer as a sequencing primer.

*KIAA1549-BRAF* fusion gene was detected using RT-PCR. Templates were prepared by amplifying first-strand cDNA using Premix Taq (Takara, Tokyo, Japan) with the forward primer for exon 15 of *KIAA1549* and a reverse primer designed to amplify exon 11 of *BRAF* as previously described[2](#_ENREF_2).

List of primers

|  |  |  |
| --- | --- | --- |
| Target gene | Forward (F)/Reverse (R) | Primer Sequence (5′–3′) |
| *TERT* promoter | F | GGCCGATTCGACCTCTCT |
| R | CAGCGCTGCCTGAAACTC |
| *IDH1* | F | TGTGGAAATCACCAAATGGCAC |
| R | TACAAGTTGGAAATTTCTGGGC |
| *IDH2* | F | GGGAGCCCATCATCTGCAAAAA |
| R | ACAAGAGGATGGCTAGGCGA |
| *H3F3A*[*1*](#_ENREF_1) | F | TCAATGCTGGTAGGTAAGTAAGGA |
| R | GGTTTCTTCACCCCTCCAGT |
| *BRAF* V600[3](#_ENREF_3) | F | TGCTTGCTCTGATAGGAAAATG |
| R | TGATGGGACCCACTCCAT |
| *KIAA1549* exon15[2](#_ENREF_2) | F | CGGAAACACCAGGTCAACGG |
| *BRAF* exon11[2](#_ENREF_2) | R | GTTCCAAATGATCCAGATCCAATTC |
| *BRAF* exon6[2](#_ENREF_2) | F | TTGTGACTTTTGTCGAAAGCTGC |
| *BRAF* exon7[2](#_ENREF_2) | R | AAGGGGATGATCCAGATGTTAGG |

Reference list

1. Aihara K, Mukasa A, Gotoh K, Saito K, Nagae G, Tsuji S, et al: H3F3A K27M mutations in thalamic gliomas from young adult patients. **Neuro Oncol 16:**140-146, 2014

2. Jones DT, Kocialkowski S, Liu L, Pearson DM, Backlund LM, Ichimura K, et al: Tandem duplication producing a novel oncogenic BRAF fusion gene defines the majority of pilocytic astrocytomas. **Cancer Res 68:**8673-8677, 2008

3. Lee D, Cho YH, Kang SY, Yoon N, Sung CO, Suh YL: BRAF V600E mutations are frequent in dysembryoplastic neuroepithelial tumors and subependymal giant cell astrocytomas. **J Surg Oncol 111:**359-364, 2015