***Supplemental Information***

***Hyperfunctioning Papillary Thyroid Carcinoma with a BRAF Mutation: The First Case Report and a Literature Review***

Shinsuke Shinkaia, Kenji Ohbaa,b, Kennichi Kakudoc, Takayuki Iwakid, Yoshihiro Mimurae, Akio Matsushitaa, Go Kurodaa, Yuki Sakaia, Nobuhiko Nishinof, Kazuo Umemurab,d, Takafumi Sudaa, Shigekazu Sasakia

aSecond Division, Department of Internal Medicine, Hamamatsu University School of Medicine, Shizuoka, Japan

bMedical Education Center, Hamamatsu University School of Medicine, Shizuoka, Japan

cDepartment of Pathology and Thyroid Disease Center, Izumi City General Hospital, Osaka, Japan

dDepartment of Pharmacology, Hamamatsu University School of Medicine, Shizuoka, Japan

eDepartment of Internal Medicine, American Hospital of Paris, Neuilly sur Seine, France

fDepartment of Surgery, Maruyama Hospital, Shizuoka, Japan

Kenji Ohba, Medical Education Center, Hamamatsu University School of Medicine, 1-20-1 Handayama, Higashi-ku, Hamamatsu 431-3192 (Japan), E-Mail ohbak@hama-med.ac.jp

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**Supplemental Methods**

**DNA extraction**

Sections measuring 10 mm × 10 mm × 80 μm were carefully excised from stored, formalin-fixed, paraffin-embedded tissues using a microtome by matching them with the hematoxylin-eosin stained images. Genomic DNA was extracted from the hyperfunctioning papillary thyroid carcinoma and the surrounding normal tissues of the gland using the Recover All Total Nucleic Acid Isolation Kit (Ambion, Foster City, CA, USA) according to the manufacturer’s protocol. The quality of the DNA samples was assessed with the help of the NanoDrop 1000 Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA), and the purity was confirmed by a 260/280 absorbance ratio greater than 1.8.

**Polymerase chain reaction (PCR) amplification and Sanger sequencing**

The PCR amplification of the target genes was performed using KOD FX DNA Polymerase (Toyobo, Osaka, Japan). The primer sequences are given in **Supplemental Table 1**. Amplification was done using a three-step touchdown protocol as follows: initial denaturation at 94°C for 2 min; one cycle at 94°C for 45 s and 72°C for 2 min; one cycle at 94°C for 45 s, 69°C for 45 s, and 72°C for 2 min; one cycle at 94°C for 45 s, 66°C for 45 s, and 72°C for 2 min; one cycle at 94°C for 45 s, 63°C for 45 s, and 72°C for 2 min; one cycle at 94°C for 45 s, 60°C for 45 s, and 72°C for 2 min; one cycle at 94°C for 45 s, 57°C for 45 s, and 72°C for 2 min; 30 cycles at 94°C for 45 s, 54°C for 45 s, and 72°C for 2 min; and a final extension step at 72°C for 4 min. The product sizes were confirmed by agarose gel electrophoresis. The amplified fragments were gel purified, extracted with phenol:chloroform, and precipitated with ethanol and Dr.GenTleR Precipitation Carrier (Takara Bio Inc., Kusatsu, Shiga, Japan). The purified products were sequenced in the forward and reverse directions using the BigDyeTM Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific, Waltham, MA, USA) and the Applied Biosystems 3130 Sequencer (Applied Biosystems, Foster City, CA, USA). The resultant electropherograms were checked by at least two independent observers using the ApE software (v2.0.61, 2020; https://jorgensen.biology.utah.edu/wayned/ape/).

**Cancer panel analysis**

Genomic mutations were analyzed using the Ion AmpliSeq Comprehensive Cancer Panel (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer’s protocol (Ion AmpliSeq Library Kit 2.0 User Guide) by the technical specialists at Macrogen Japan Corp., Kyoto, Japan.

**Immunohistochemistry**

Immunohistochemical analyses were performed using antibodies against BRAFV600E (clone VE1, Spring Bioscience, Pleasanton, CA, USA) and thyroglobulin (rabbit antihuman thyroglobulin, Dako, Glostrup, Denmark) by the technical specialists at Kyodo Byori, Kobe, Hyogo, Japan.

**Supplemental Table 1.** PCR primer sequences.

|  |  |  |  |
| --- | --- | --- | --- |
| Gene | Primer sequence | Amplicon size (bp) | Reference |
| *BRAF* (exon 15) | 5'-ACATACTTATTGACTCTAAGAGGAAAGATGAA-3' | 400 | [1] |
|  | 5'-GATTTTTGTGAATACTGGGAACTATGA-3' |  |  |
| *EZH1* (exon 16) | 5'-TCCCTTCCCAGGTCAGAATC-3' | 224 | [2] |
|  | 5'-TTATGTGGTGTGGGGTCCTG-3' |  |  |
| *GNAS* (exon 7) | 5'-TGCTGCATAACTGTGGGACG-3' | 225 | [3] |
|  | 5'-GTAGTTTGGAAAGAGGGCTCAG-3' |  |  |
| *GNAS* (exon 8, 9) | 5'-TGTTTCGGTTGGCTTTGGTG-3' | 348 |  |
|  | 5'-AGCGACCCTGATCCCTAACA-3' |  |  |
| *GNAS* (exon 10) | 5'-TGTTAGGGATCAGGGTCGCTG-3' | 234 | [3] |
|  | 5'-AACAGTGCAGACCAGGGCCTCCTG-3' |  |  |
| *HRAS* (codon 12, 13) | 5'-AGCAGGGCCCTCCTTGGCAG-3' | 159 |  |
|  | 5'-CACCTCTATAGTGGGGTCGTATTC-3' |  |  |
| *HRAS* (codon 61) | 5'-GGAGACCCTGTAGGAGGACCC-3' | 298 | [1] |
|  | 5'-CCACCTGTGCGGCGTGGGCT-3' |  |  |
| *KRAS* (codon 12, 13) | 5'-GGTACTGGTGGAGTATTTGATAGT-3' | 290 | [1] |
|  | 5'-CTCATGAAAATGGTCAGAGAAACCT-3' |  |  |
| *KRAS* (codon 61) | 5'-CTGTGTTTCTCCCTTCTCAGG-3' | 276 | [3] |
|  | 5'-GGCATTAGCAAAGACTCAAAAA-3' |  |  |
| *NRAS* (codon 12, 13) | 5'-CACACTAGGGTTTTCATTTCCATTG-3' | 283 | [1] |
|  | 5'-GGTAAAGATGATCCGACAAGTGAG-3' |  |  |
| *NRAS* (codon 61) | 5'-AGCATTGCATTCCCTGTGGT-3' | 331 | [3] |
|  | 5'-GGTAAAGATGATCCGACAAGTGAG-3' |  |  |
| *TERT* promoter | 5'-CTGCCCCTTCACCTTCCAG-3' | 159 |  |
|  | 5'-AGCGCTGCCTGAAACTCG-3' |  |  |
| *TSHR* (exon 1) | 5'-TCTCCTTTGGCCTGGGGTAA-3' | 353 |  |
|  | 5'-TCGGGCTGTTATTGAGCTGC-3' |  |  |
| *TSHR* (exon 2) | 5'-TCAGCCAACATATTGTGAAAACTGT-3' | 299 |  |
|  | 5'-ACTGCCATTGATTTATGCAAGTAT-3' |  |  |
| *TSHR* (exon 3) | 5'-GAGGGTTGTACATGTTGCATGA-3' | 268 |  |
|  | 5'-CTGGAGCCCCAAGATTATCAGT-3' |  |  |
| *TSHR* (exon 4) | 5'-GGTACCCTGTGGCGTAAATG-3' | 330 |  |
|  | 5'-CGACCCAGGCTATACACCAT-3' |  |  |
| *TSHR* (exon 5) | 5'-AGGTGTTGGGAGTTTGACTACA-3' | 337 |  |
|  | 5'-GTCACACCTGATCATTGCTTACTC-3' |  |  |
| *TSHR* (exon 6) | 5'-AGGGTCAGTGAAACTTAAAAAGAAA-3' | 298 |  |
|  | 5'-TGGCTCTTGGATGGTCTGTAAA-3' |  |  |
| *TSHR* (exon 7) | 5'-TCCAACTCTCCAGAAACAGGC-3' | 262 |  |
|  | 5'-TCACACTAACTCTGGCGTGG-3' |  |  |
| *TSHR* (exon 8) | 5'-TTAAGTGCTCAAGCCAGAAGAAGA-3' | 370 |  |
|  | 5'-TTGCAGAAGCCTTTACCCGAG-3' |  |  |
| *TSHR* (exon 9) | 5'-CATCTCCCAATTAACCTCAGGC-3' | 381 | [3] |
|  | 5'-CAAACCAGGAAGCATCTTCCC-3' |  |  |
| *TSHR* (exon 10) | 5'-CAGCCTGGCACTGACTCTTT-3' | 263 |  |
|  | 5'-AGCTCCTGGCCAAAACCAAT-3' |  |  |
|  | 5'-CCTTGAATAGCCCCCTCCAC-3' | 264 |  |
|  | 5'-ACTCATCGGACTTGGGGGTA-3' |  |  |
|  | 5'-GAGCTCAAAAACCCCCAGGA-3' | 197 |  |
|  | 5'-AAGACATTGCCCAGGAGAGC-3' |  |  |
|  | 5'-TCCGATGAGTTCAACCCGTG-3' | 162 |  |
|  | 5'-CCCGCTTTCTCATGTGCAAC-3' |  |  |
|  | 5'-GCTCTCCTGGGCAATGTCTT-3' | 377 |  |
|  | 5'-GTTTGCTGCTTCCTTCTCGC-3' |  |  |
|  | 5'-TGCAAGCGAGTTATCGGTGT-3' | 339 |  |
|  | 5'-AATCCGCAGTACAACCCAGG-3' |  |  |
|  | 5'-ACATAGTTGCCTTCGTCATCGT-3' | 380 | [3] |
|  | 5'-GCTGTTCTTTGGAGGAACCC-3' |  |  |
|  | 5'-GCCTTCCAGAGGGATGTGTTC-3' | 381 | [3] |
|  | 5'-CCATGAAACATTGAAACATCGC-3' |  |  |

Primer pairs devoid of references were designed in-house using NCBI-Primer-BLAST (https://www.ncbi.nlm.nih.gov/tools/primer-blast/).

**Supplemental Table 2.** Literature review on the clinical and molecular characteristics of hyperfunctioning carcinomas.

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| No. | Age  (year) | Sex | Size5)  (cm) | Histological  diagnosis | Thyroid  function | *BRAF* | *RAS* | *TSHR* | *GNAS* | Reported  year |
| 1 | 60 | F | 6.0 | ITC | OH | NA | WT | D633H | NA | 1997 [4] |
| 2 | 42 | F | NA | HCC | SCH | NA | WT | L677V | WT | 1999 [5] |
| 3 | 11 | F | 4.0 | PTC | OH | NA | NA | M453T | NA | 2000 [6] |
| 4 | 49 | F | 3.5 | FTC | OH | NA | NA | F486I | NA | 2000 [7] |
| 51) | 59 | M | 5.0 | FTC | SCH | NA | WT | D633Y | NA | 2003 [8] |
| 62) | 59 | M | 2.0 | FTC | SCH | NA | WT | F631I | NA | 2003 [8] |
| 7 | NA | F | 5.0 | PTC | OH | NA | NA | L512R | WT | 2004 [9] |
| 8 | 64 | F | 6.0 | FTC | OH | NA | kG12C | T620I | NA | 2006 [10] |
| 93) | 59 | F | 5.0 | FTC | OH | WT | WT | M453T | NA | 2010 [11] |
| 104) | 15 | F | 3.5 | fvPTC | SCH | WT | WT | PM | PM | 2013 [12] |
| 11 | 29 | F | 2.7 | miFTC | SCH | WT | WT | NA | NA | 2013 [13] |
| 12 | 14 | F | 3.7 | PTC | OH | WT | WT | WT | WT | 2014 [14] |
| 13 | 16 | F | 4.2 | FTC | OH | WT | WT | WT | WT | 2014 [14] |
| 14 | 17 | M | 4.2 | PTC | SCH | WT | WT | T632I | WT | 2014 [14] |
| 15 | 48 | M | 3.7 | FTC | OH | WT | WT | F631L | WT | 2018 [15] |
| 16 | 64 | M | 4.3 | FTC | OH | WT | WT | I568F | WT | 2018 [15] |
| 17 | 48 | M | 2.6 | PTC | OH | V600E | WT | D727E  PM | WT | Current  study |

1), 2) indicate nodule 1 and nodule 2 in the same patient, respectively. 3) indicates that the patient has a *PAX8/PPARG* rearrangement mosaicism. 4) indicates that the nodule has a coexisting polymorphism in *TSHR* and *GNAS*. 5) indicates the maximum size determined by palpation, ultrasonography, or the resected specimen.

Abbreviations: NA, not available; M, male; F, female; ITC, insular thyroid carcinoma; HCC, Hürthle cell carcinoma; PTC, papillary thyroid carcinoma; FTC, follicular thyroid carcinoma; fvPTC, follicular variant of PTC; miFTC, minimally invasive FTC; OH, overt hyperthyroidism; SCH, subclinical hyperthyroidism; kG12C, *KRAS* G12C; PM, polymorphism.

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