doi: 10.2176/nmc.rc.2020-0339

Neurol Med Chir (Tokyo) 61, 385–391, 2021

Online May 8, 2021

Craniopharyngiomas, including Recurrent Cases, *Lack TERT Promoter Hotspot Mutations*

Shingo Fujio,[1,2,3,4] Tareq A. Juratli,[1,2,5] Tomoko Takajo,[3] Kazunori Arita,[3] Yushi Nagano,[3] Koji Yoshimoto,[3,4] Naema Nayyar,[1] William T. Curry Jr,[2] Maria Martinez-Lage,[6] Daniel P. Cahill,[2,*] Fred G. Barker II,[2,*] and Priscilla K. Brastianos[1,*]

[1]*Divisions of Neuro-Oncology and Hematology/Oncology, Departments of Medicine and Neurology, Massachusetts General Hospital Cancer Center, Harvard Medical School, Boston, MA, USA*

[2]*Department of Neurosurgery, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA*

[3]*Department of Neurosurgery, Graduate School of Medical and Dental Sciences, Kagoshima University, Kagoshima, Kagoshima, Japan*

[4]*Pituitary Disorders Center, Kagoshima University Hospital, Kagoshima, Kagoshima, Japan*

[5]*Department of Neurosurgery, Faculty of Medicine and Carl Gustav, Carus University Hospital, Technische Universität Dresden, Dresden, Germany*

[6]*Department of Pathology, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA*

Abstract

Adamantinomatous craniopharyngiomas (ACP) are characterized by alterations in the *CTNNB1* gene while almost all papillary craniopharyngiomas (PCP) harbor a canonical *V600E* mutation in the BRAF gene. Although other recurrent driver genes have not been described to date in craniopharyngiomas, the heterogeneous clinical course of these tumors might be associated with the acquisition of further genomic alterations. It is well known that telomerase reverse transcriptase (*TERT*) promoter (*TERT*p) alterations, including mutations or methylation, upregulate the expression of *TERT* and increase telomerase activity, promoting tumorigenesis. We investigated whether *TERT*p mutations or methylation are associated with tumor relapse in a subset of craniopharyngiomas. Samples from 42 patients with histologically confirmed craniopharyngioma were retrieved. We determined *TERT*p, *BRAF*, and *CTNNB1* hotspot mutations in all samples using targeted sequencing and the *TERT*p methylation status by methylation-specific polymerase chain reaction (PCR) in 30 samples. While *BRAF* V600E mutations and *CTNNB1* mutations were detected in 12 (28.6%) and 21 patients (50%) in the initial tumors and subsequent recurrences, respectively, none of the patients in our cohort, including those with multiple relapses, harbored a *TERT*p mutation. Furthermore, *TERT*p methylation was detected in 14 out of 24 cases (58.3%) with available primary samples; however, no correlation between *TERT*p methylation with the pathological subtype, genotype, or tumor aggressiveness was detected. These data suggest that elevated telomerase activity via acquisition of *TERT*p mutations is an infrequent pathway in the tumorigenesis of craniopharyngiomas, regardless of their clinical course.

Keywords: BRAF, craniopharyngiomas, CTNNB1, methylation, TERT

Received September 27, 2020; Accepted January 25, 2021

* These authors are Senior author.

Introduction

Craniopharyngiomas are rare primary brain tumors originating from the ectopic embryonic remnants of the craniopharyngeal duct or squamous epithelial cells.[1–3] Although histologically defined as benign tumors, craniopharyngiomas often compress nearby critical structures, such as the optic nerves, the pituitary gland, or the hypothalamus.[1,2,4,5] There are two histological subtypes of craniopharyngioma: adamantinomatous craniopharyngioma (ACP) and papillary craniopharyngioma (PCP). ACP are genomically characterized by alterations of the *CTNNB1* gene while almost all PCP harbor canonical V600E mutations in the *BRAF* gene which constitutively activates the mitogen-activated protein kinase signaling pathway.[1,6] Although further recurrent driver genes in craniopharyngioma have not been described to date, the heterogeneous clinical course of these tumors might be associated with the acquisition of further genomic alterations. It is well known that telomerase reverse transcriptase (*TERT*) promoter mutations upregulate the *TERT* expression and increase telomerase activity, resulting in tumorigenesis. *TERT* promoter mutations are frequent in progressive and aggressive brain tumors, including glioblastoma and high-grade meningioma.[7,8] In addition to *TERT* promoter mutations, *TERT* promoter methylation was shown to be frequent in a series of central nervous system tumors and was reported to be associated with a worse prognosis in cases with increased *TERT* expression.[9,10]

With this in mind, we investigated whether *TERT* promoter mutations and methylation could be detected in a subset of craniopharyngiomas, in particular, tumors with multiple relapses.

Patients and Methods

Patient's characteristics

With institutional review board approval, samples from 42 patients who underwent surgery and were histologically diagnosed with craniopharyngioma were retrieved. The cohort comprised 17 men and 25 women, with ages ranging from 0.8 to 77 years (median age at diagnosis was 44 years). Our cohort included 10 pediatric patients aged <18 years (23.8%). In 13 patients who underwent repeat surgery, six had a recurrent tumor with available specimens to be analyzed.

Tissue samples and DNA extraction

Tissue samples were formalin-fixed and paraffin-embedded (FFPE). The DNA for genetic analysis was extracted from a tissue shaving or punch of FFPE tissue, using a QIAamp DNA FFPE Tissue kit (Qiagen, Germantown, MD, USA), according to the manufacturer's instructions.

Genetic analysis

Polymerase chain reaction (PCR) amplification was carried out using the following primers: TERT forward: 5′GTCCTGCCCCTTCACCTT3′, TERT reverse: 5′CAGCGCTGCCTGAAACTC3′, BRAF forward: 5′CAGACAACTGTTCAAACTGATGGGACCCAC3′, BRAF reverse: 5′TGCTTGCTCTGATAGGAAAATGA3′, and CTNNB1 forward: 5′AGTTGGACATGGCCATGGAA3′, CTNNB1 reverse: 5′ACATCCTCTTCCTCAGGATT3′. Subsequently, Sanger sequencing was performed to identify the mutations.

Methylation-specific PCR

In all, 30 samples with a sufficient remaining amount from 26 patients were analyzed by methylation-specific (MS)-PCR. The DNA was extracted from a tissue using the DNeasy Blood & Tissue Kit (Qiagen) and subjected to bisulfite conversion (EpiTect Fast DNA Bisulfite Kit, Qiagen). MS-PCR amplification was carried out using the following primer pairs: forward-primer, 5′GGGAAGTGTTG TAGGGAGGTATTT (methylated and unmethylated); reverse-primer, 5′CGTACGACGACCCTTTAACCG (methylated); and reverse-primer, 5′CATACAACAACCCTTTAACCA (unmethylated) modifying Köchling's report.[11]

Results

None of the patients in our cohort harbored a *TERT* promoter mutation. Table 1 summarizes the genetic profiles of cases. In six recurrent cases with available specimens, five were recurrent or distant tumors after radiation. *TERT* promoter mutations were not detected in any of the local or distant recurrent tumors. *TERT* promoter methylation was detected in 14 out of 24 cases (58.3%) with available primary samples (Table 1). No correlation was found between the methylation status and the pathological subtype, genotype, or tumor aggressiveness. *TERT* promoter methylation was detected in three of the six pediatric cases whose initial tumor samples were analyzed.

On the other hand, in accordance with previous studies, we detected the *BRAF* V600E mutation in 12 of the 42 patients (28.6%). All patients with *BRAF* mutations had a histologic diagnosis of a PCP. In addition, *CTNNB1* mutations were found in 21 patients (50%). All patients with the *CTNNB1* mutation were diagnosed with ACP. These mutations were mutually exclusive. In 9 of the 42 patients, neither mutation could be detected. Of the two

Table 1 Genetic profiles of craniopharyngioma cases

Case	Age (years)	Sex	Pathological subtype	BRAF V600E	CTNNB1	TERTp	TERTp methylation	Operation	Status
1	19	Female	Adamantinomatous	WT	WT	WT	Methylated	First	
2	65	Male	Papillary	Mutated	WT	WT	n/a	First	
3	20	Female	Adamantinomatous	WT	WT	WT	Unmethylated	First	
4	72	Male	Papillary	WT	WT	WT	n/a	First	
5	77	Male	Adamantinomatous	WT	Mutated	WT	Unmethylated	First	
6	70	Male	Papillary	Mutated	WT	WT	n/a	First	
7	39	Male	Adamantinomatous	WT	Mutated	WT	Methylated	First	
8	18	Female	Papillary	WT	WT	WT	n/a	First	
9	72	Female	Adamantinomatous	WT	WT	WT	Methylated	First	
10	47	Female	Adamantinomatous	WT	WT	WT	n/a	First	
11	58	Female	Papillary	Mutated	WT	WT	n/a	Second	Staged surgery
12	34	Male	Papillary	Mutated	WT	WT	n/a	First	
		Male	Papillary	Mutated	WT	WT	n/a	Second	Staged surgery
		Male	Papillary	Mutated	WT	WT	Unmethylated	Third	Recurrence after radiation
13	29	Female	Adamantinomatous	WT	Mutated	WT	Methylated	First	
14	76	Male	Adamantinomatous	WT	Mutated	WT	Methylated	First	
15	29	Female	Adamantinomatous	WT	Mutated	WT	Unmethylated	First	
			Adamantinomatous	WT	n/a	WT	n/a	Second	Recurrence after radiation
16	50	Female	Papillary	WT	WT	WT	Methylated	First	
			Papillary	WT	WT	WT	n/a	Second	Staged surgery
17	49	Female	Papillary	Mutated	WT	WT	Methylated	First	
18	6	Female	Adamantinomatous	WT	Mutated	WT	Unmethylated	First	
19	62	Female	Adamantinomatous	WT	Mutated	WT	Unmethylated	First	
			Adamantinomatous	WT	Mutated	n/a	n/a	Second	Recurrence
			Adamantinomatous	WT	Mutated	WT	Methylated	Third	Recurrence after radiation
20	3	Female	Adamantinomatous	WT	Mutated	n/a	Unmethylated	First	
			Adamantinomatous	WT	Mutated	n/a	Unmethylated	Second	Staged surgery
			Adamantinomatous	WT	Mutated	WT	Methylated	Third	Distant recurrence after radiation
21	4	Female	Adamantinomatous	WT	Mutated	WT	Methylated	First	
22	18	Male	Papillary	Mutated	WT	WT	Methylated	First	
23	33	Male	Adamantinomatous	WT	Mutated	WT	Methylated	First	
24	10	Male	Adamantinomatous	WT	Mutated	WT	n/a	First	
25	61	Female	Adamantinomatous	WT	Mutated	WT	n/a	First	
26	19	Female	Adamantinomatous	WT	Mutated	WT	Unmethylated	First	
27	44	Male	Adamantinomatous	WT	Mutated	WT	n/a	Second	Staged surgery

Table 1 Continued

Case	Age (years)	Sex	Pathological subtype	BRAF V600E	CTNNB1	TERTp	TERTp methylation	Operation	Status
28	55	Female	Papillary	Mutated	WT	WT	Methylated	First	
			Papillary	Mutated	WT	n/a	n/a	Second	Recurrence
			Papillary	Mutated	WT	n/a	Unmethylated	Third	Recurrence after radiation
29	46	Female	Adamantinomatous	WT	Mutated	WT	n/a	First	
30	58	Female	Adamantinomatous	WT	Mutated	WT	n/a	First	
31	10	Male	Adamantinomatous	WT	WT	WT	Methylated	First	
32	2	Female	Adamantinomatous	WT	Mutated	WT	Methylated	First	
33	17	Male	Adamantinomatous	WT	Mutated	WT	n/a	First	
34	44	Male	Papillary	Mutated	WT	WT	n/a	First	
35	55	Male	Papillary	Mutated	WT	WT	Unmethylated	First	
36	48	Male	Papillary	Mutated	WT	WT	n/a	First	
37	51	Female	Adamantinomatous	WT	Mutated	WT	Unmethylated	First	
38	7	Female	Adamantinomatous	WT	Mutated	WT	n/a	First	
39	55	Male	Papillary	Mutated	WT	WT	Unmethylated	Second	Recurrence
40	48	Female	Papillary	Mutated	WT	WT	Methylated	First	
41	0	Female	Adamantinomatous	WT	WT	WT	Unmethylated	First	
42	14	Female	Adamantinomatous	WT	Mutated	WT	n/a	First	

n/a: not applicable, TERTp: telomerase reverse transcriptase promoter, WT: wild type.

recurrent *BRAF*-V600E-mutated cases (Cases 12 and 28) where tissue was available from more than one surgery, *BRAF* V600E mutations were detected in both the initial resection and the subsequent surgery. Likewise, in the two recurrent *CTNNB1*-mutated cases (Cases 19 and 20) with available tissue from both surgeries, *CTNNB1* mutations were consistently detected in the initial as well as in the consecutive tumor.

A representative case of a craniopharyngioma with an aggressive clinical course

Case 12

A 34-year-old man presented to our hospital with visual deterioration. Magnetic resonance imaging (MRI) showed a mainly solid tumor extending to the 3rd ventricle (Figs. 1a and 1b). The tumor was partially removed by staged surgeries via right and left pterional approaches. The histologic diagnosis revealed a PCP. One month later, we performed Gamma Knife radiosurgery for residual tumor (marginal dose 16 Gy). Six years later, the tumor relapsed (Figs. 1c and 1d), and we performed a third surgery via initially a transsphenoidal approach, but the tumor was strongly adherent to the optic chiasm, and the surgery was completed through partial excision. This was followed with conventional local 40 Gy irradiation for residual tumor. Eventually, the tumor was controlled, but the patient had residual mild neurocognitive deficits. While a *BRAF V600E* mutation was detected in the initial tumor and subsequent recurrences, no *CTNNB1* nor *TERT* promoter mutations were present in any of the specimens. Moreover, the *TERT* promoter methylation assay demonstrated an unmethylated status in the sample from the recurrent tumor after radiation.

Case 20

A 3-year-old girl was referred to our hospital with a change in mental status. MRI showed a mainly cystic tumor occupying the 3rd ventricle with hydrocephalus (Figs. 2a and 2b). The tumor was subtotally removed by staged surgeries via transcortical-transventricular and pterional approaches (Figs. 2c and 2d). The histologic evaluation revealed ACP. Three months later, Gamma Knife radiosurgery was performed for the residual tumor (marginal dose 14 Gy). Two years later, the tumor relapsed dorsally to the anterior commissure, for which we performed Gamma Knife radiosurgery (marginal dose 14 Gy). Four years later, the tumor relapsed on the body of the lateral ventricle, which appeared to be a distant recurrence associated with the initial surgery (Figs. 2e and 2f) and removed via a

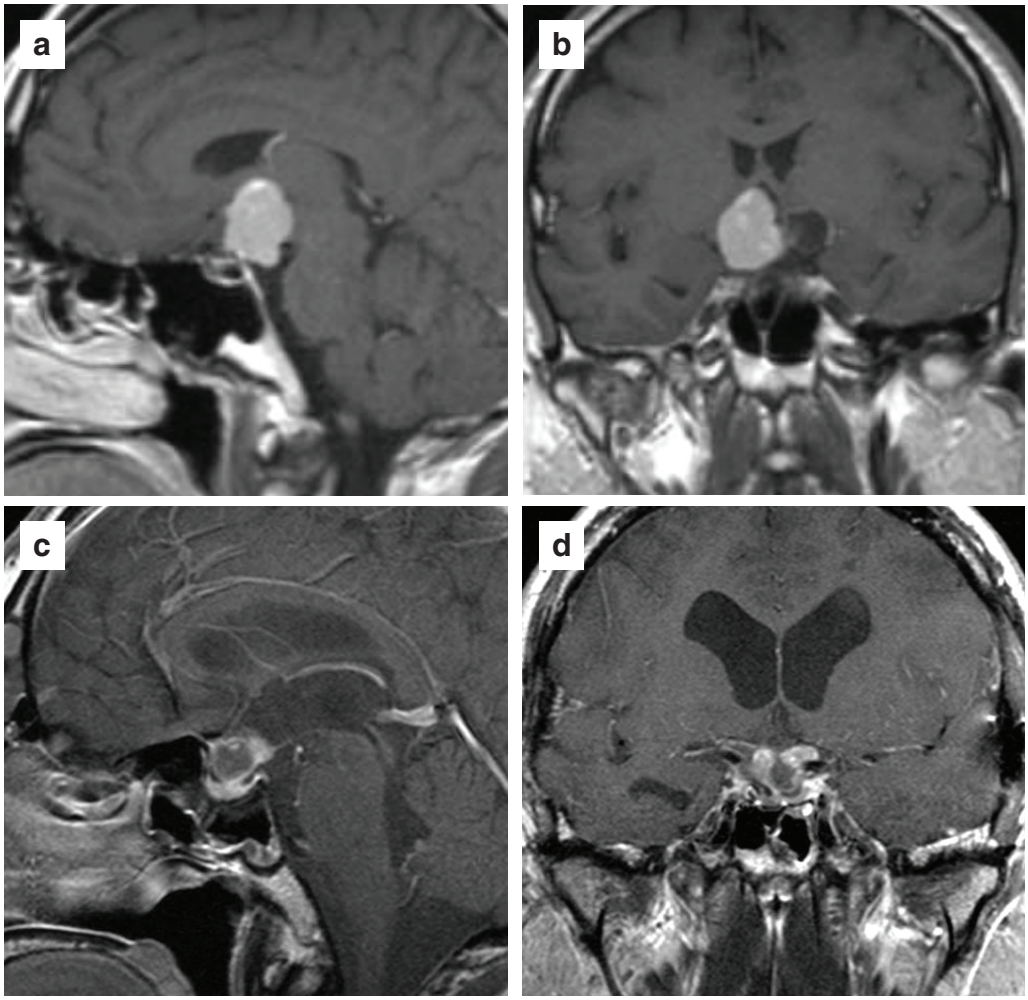

Fig. 1 Case 12: A 34-year-old man. Preoperative sagittal (a) and coronal (b) enhanced T1-weighted MRI shows a mainly solid tumor extending to the 3rd ventricle. Sagittal (c) and coronal (d) enhanced T1-weighted MRI performed 6 years after Gamma Knife radiosurgery demonstrate the tumor relapse in the sella. MRI: magnetic resonance imaging.

transcortical approach. At 5 years, there is no evidence for tumor recurrence. While a *CTNNB1* mutation was detected in the initial tumor and in the subsequent recurrences, no *BRAF* mutations were present in any of the specimens. For the *TERT* promoter mutation assessment, only the tissue from the distant recurrence was available, with no mutations detected. *TERT* promoter methylation was not observed in the first and second surgeries, but interestingly, methylation was detected in the distant recurrence.

Discussion

In our study, we performed targeted sequencing of *TERT* promoter, *BRAF* and *CTNNB1* hotspot mutations in a large cohort of 42 craniopharyngiomas. Notwithstanding, none of the tumors in our cohort, although enriched with recurrent cases, harbored a *TERT* hotspot mutation in the promoter region, regardless of their *BRAF* and *CTNNB1* mutation status. This is consistent with a prior study by Koelsche and colleagues who found no *TERT* promoter mutations in a small cohort of craniopharyngiomas.[12] Based on the results of Koelsche and our study, we assume that elevated telomerase activity via acquisition of *TERT* promoter mutations is not a frequent pathway in craniopharyngioma, regardless of their clinical course or molecular subtype.

TERT promoter mutations in tumors of the sellar region are extremely rare.[12] However, Miyake et al.[10] showed that *TERT* promoter methylation is associated with disease progression in pituitary adenomas. Pediatric tumors of the nervous system rarely exhibit *TERT* promoter mutations.[12] Our cohort included 10 pediatric cases, and there were no *TERT* promoter mutations in these cases. On the other hand,

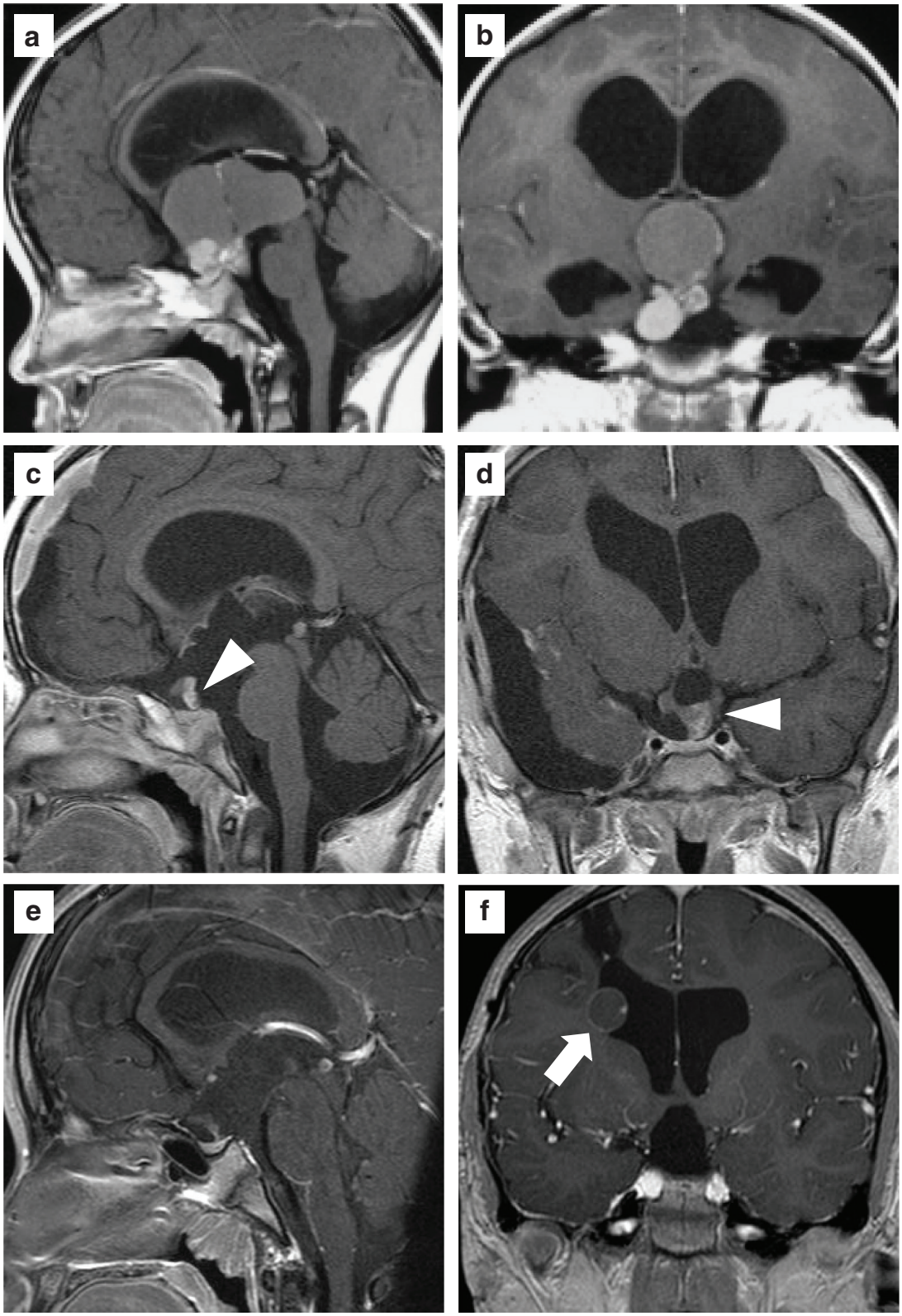

Fig. 2 Case 20: A 3-year-old girl. Preoperative sagittal (a) and coronal (b) enhanced T1-weighted MRI shows a mainly cystic tumor occupying the 3rd ventricle with hydrocephalus. Postoperative sagittal (c) and coronal (d) enhanced T1-weighted MRI demonstrate the residual tumor behind the optic chiasm (arrow head). Sagittal (e) and coronal (f) enhanced T1-weighted MRI performed 6 years after the initial surgery demonstrate distant recurrence in the body of the lateral ventricle (arrow). MRI: magnetic resonance imaging.

TERT promoter methylation was not infrequently observed in both adults and pediatric patients in our cohort. To our knowledge, our study is the first to investigate *TERT* promoter methylation in craniopharyngiomas. However, there was no association between *TERT* promoter methylation and pathological subtype, genotype, or tumor aggressiveness. It has been reported that the methylation status of *TERT* is associated with *TERT* expression and progression in childhood brain tumors, especially malignant tumors.[9] As *TERT* promoter methylation was not associated with clinical outcomes in our study, we cannot make any conclusions about *TERT* promoter methylation and clinical status in craniopharyngiomas. Further investigations are needed to clarify the role of *TERT* promoter methylation, and how radiation may affect *TERT* promoter methylation in craniopharyngiomas.

To date, little is known about the oncogenic drivers of craniopharyngiomas other than *CTNNB1* and *BRAF*. In cases where we had recurrent tumor samples, mutations of *BRAF* or *CTNNB1* were present in the initial and subsequent recurrences, which suggests that mutations in *BRAF* or *CTNNB1* are early events in craniopharyngioma evolution and persist at recurrence. To elucidate the factors that may influence tumorigenesis and biological behavior of craniopharyngiomas, future studies, including epigenetic analyses, are needed.

Funding

This work is supported by the Burroughs Wellcome Fund Career Award (to D.P.C.). P.K.B. is also supported by the Damon Runyon Cancer Research Foundation and the Breast Cancer Research Foundation.

Conflicts of Interest Disclosure

Dr Brastianos has consulted for Angiochem, Genentech-Roche, Lilly, Tesaro, ElevateBio, Pfizer (Array), Dantari, SK Life Sciences, Voyager Therapeutics, received grant/research support to MGH from Merck, BMS, Mirati and Lilly and honoraria from Merck, Genentech-Roche, Pfizer and Lilly. Dr Cahill has consulted for Lilly, GlaxoSmithKline, and Boston Pharmaceuticals, and has received honoraria and travel reimbursement from Merck for invited lectures, and from the US NIH and DOD for clinical trial and grant review. The authors have no personal, financial, or institutional interest in any of the drugs, materials, or devices described in this article. All authors who are members of The Japan Neurological Society (JNS) have registered online self-reported COI Disclosure Statement Forms through the JNS member website.

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Corresponding author: Priscilla K. Brastianos, MD
Stephen E. and Catherine Pappas Center for Neuro-Oncology, Divisions of Neuro-Oncology and Hematology/Oncology, Departments of Medicine and Neurology, Massachusetts General Hospital Cancer Center, Harvard Medical School, 55 Fruit Street, Boston, Massachusetts 02114, USA.
e-mail: PBRASTIANOS@mgh.harvard.edu