



# Identification of a novel *KLC1–ROS1* fusion in a case of pediatric low-grade localized glioma

Yoshiko Nakano<sup>1,2</sup> · Arata Tomiyama<sup>1,3</sup> · Takashi Kohno<sup>4</sup> · Akihiko Yoshida<sup>5</sup> · Kai Yamasaki<sup>1,2</sup> · Tatsuya Ozawa<sup>1</sup> · Kohei Fukuoka<sup>1</sup> · Hiroko Fukushima<sup>6</sup> · Takeshi Inoue<sup>6</sup> · Junichi Hara<sup>2</sup> · Hiroaki Sakamoto<sup>7</sup> · Koichi Ichimura<sup>1</sup>

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## Abstract

The proto-oncogene tyrosine-protein kinase ROS1 (ROS1) is a tyrosine kinase that is closely related to anaplastic lymphoma kinase receptor (ALK). We describe a novel *KLC1–ROS1* fusion identified in a case of pediatric low-grade glioma. This was detected by RNA sequencing and confirmed by reverse-transcription PCR and fluorescent in situ hybridization. Immunohistochemical staining for ROS1 was positive in the tumor cytoplasm. In vitro analysis demonstrated the oncogenic activity of this fusion, which was suppressed by the ALK/ROS1 inhibitor, crizotinib. Our case and others suggest that various ROS1 fusions might be present in a subset of pediatric gliomas, which could be targeted for therapy.

**Keywords** Crizotinib · Immunohistochemistry · FISH · *KLC1–ROS1* fusion · Pediatric glioma

## Introduction

*ROS1*, located on chromosome 6, encodes the proto-oncogene ROS1 [1, 2]. As an orphan receptor tyrosine kinase, it is closely related to anaplastic lymphoma kinase (ALK). Both have an extracellular domain, a transmembrane region,

and an intracellular tyrosine kinase domain, and are involved in the activation of the JAK/STAT, RAS/MAPK, and PI3K pathways. The first *ROS1* fusion, *GOPC–ROS1*, was discovered in a human glioblastoma cell line (U118MG). Since then, more than 25 *ROS1* fusions with various 5' fusion partners have been detected in diverse types of cancers including non-small lung cancer and cholangiocarcinomas; however, they are not often found in clinical samples of adult glioblastoma [1, 3]. *ROS1* fusion genes invariably retain the kinase domain of ROS1. The important clinical implication of *ROS1* fusions is that they can be targeted by ROS1 inhibitors [2]. Here, we present a novel *KLC1–ROS1* fusion identified in a case of pediatric low-grade glioma. In vitro analysis demonstrated that the fusion has oncogenic activity that can be suppressed by an ROS1 inhibitor.

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✉ Yoshiko Nakano  
yonakano@ncc.go.jp

<sup>1</sup> Division of Brain Tumor Translational Research, National Cancer Center Research Institute, 5-1-1, Tsukiji, Chuo-ku, Tokyo 104-0045, Japan

<sup>2</sup> Department of Pediatric Hematology/Oncology, Osaka City General Hospital, Osaka, Japan

<sup>3</sup> Department of Neurosurgery, National Defense Medical College, Saitama, Japan

<sup>4</sup> Division of Genome Biology, National Cancer Center Research Institute, Tokyo, Japan

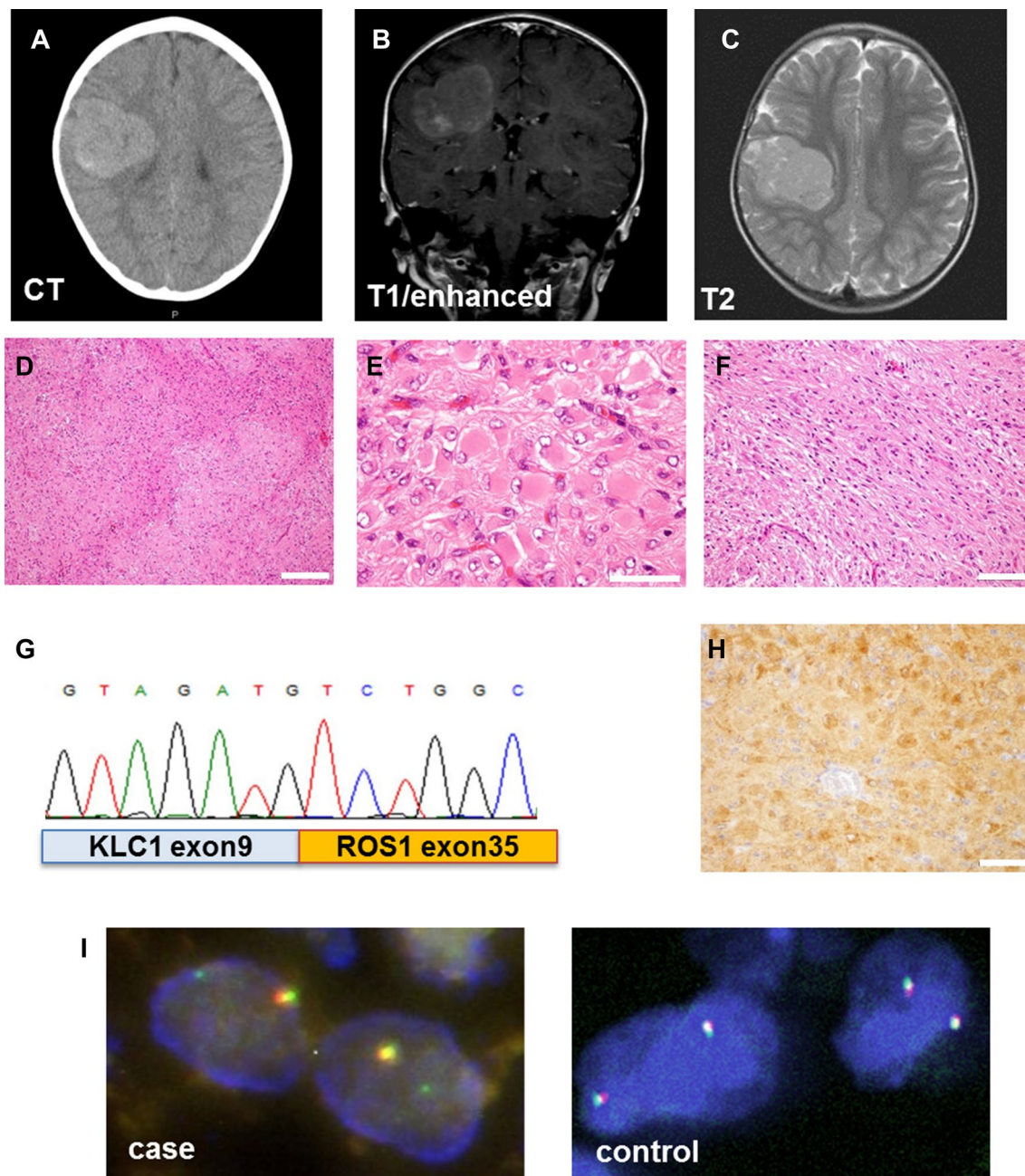
<sup>5</sup> Department of Pathology and Clinical Laboratories, National Cancer Center Hospital, Tokyo, Japan

<sup>6</sup> Department of Pathology, Osaka City General Hospital, Osaka, Japan

<sup>7</sup> Department of Pediatric Neurosurgery, Osaka City General Hospital, Osaka, Japan

## Clinical summary

A 3-year-old girl was suspected of having a brain tumor when she received a computed tomography scan after a minor head injury (Fig. 1a). The patient was neurologically asymptomatic. Magnetic resonance imaging showed a slightly gadolinium-enhanced mass (5.7 × 4.0 × 3.8 cm) located in the right frontal lobe (Fig. 1b, c). The patient underwent a gross total tumor resection and experienced no recurrence for 7 years.



**Fig. 1** Clinical and histopathological features of pediatric glioma patient and detection of *KLC1-ROS1* fusion. **a–c** Computed tomography (CT) and magnetic resonance imaging (MRI) demonstrated a partially enhanced mass in the right frontal lobe. Histologically, the tumor showed vague internal multinodularity (**d**) and was composed of cellular astrocytic proliferation embedded within a dense compact background of glial filaments. Most tumor cells were gemistocytic in appearance (**e**), whereas a minor subset of cells was of fibrillary and spindle-type (**f**). Hematoxylin and eosin stain. Magnifications

and scale bars are  $\times 100$ ,  $200 \mu\text{m}$ ,  $\times 600$ ,  $50 \mu\text{m}$ , and  $\times 200$ ,  $50 \mu\text{m}$  respectively (for **d–f**). **g** *KLC1-ROS1* fusion was validated by Sanger sequencing of the PCR product. **h** Immunohistochemical analysis showed diffuse cytoplasmic ROS1 protein expression ( $\times 400$  magnification; scale bar =  $50 \mu\text{m}$ ). **i** Fluorescent in situ hybridization analyses using ROS1 break-apart probes showed an isolated green (3') signal in addition to the intact red/green fused signal, indicating an *ROS1* rearrangement (left). Two red/green fused signals per nucleus were detected in the fusion-negative control (right)

### Pathological findings

The tumor was sharply demarcated from the surrounding brain tissue. It showed vague internal multinodularity

and was composed of cellular astrocytic proliferation embedded within a dense compact background of glial filaments (Fig. 1d). Most tumor cells were gemistocytic in appearance and harbored relatively uniform, vesicular,

non-hyperchromatic nuclei (Fig. 1e). A minor subset of cells was fibrillary and spindle type (Fig. 1f). Mitotic figures were not observed. No dysmorphic ganglion cells were present, nor was there morphological evidence of ependymal differentiation. No necrosis or microvascular proliferation was present. Rosenthal fibers and eosinophilic granular bodies were not observed. There was a focal area where numerous minute calcospherites were deposited. Immunohistochemically, the tumor cells were positive for glial fibrillary acidic protein, S100 protein, synaptophysin (focal, dot-like), Olig2 (weak, focal), epithelial membrane antigen (focal, dot-like), p53 (focal, wild-type pattern), and ATRX (retained), whereas they were negative for CD34. No CD34-positive ramifying elements were observed. The MIB-1 labeling index was approximately 4.3%. The non-infiltrative nature of the tumor was highlighted by the lack of transgressing neurofilament-positive fibers within the tumor tissue.

## Molecular analysis

### Identification of *KLC1–ROS1* fusion

Targeted sequencing of all coding exons of 93 selected brain tumor-related genes using DNA isolated from the tumor sample revealed no significant somatic pathogenic variants (Supplementary Information S1 and Supplementary Table S1). We then performed RNA sequencing (Supplementary Information S1) and a *KLC1–ROS1* fusion was detected. The presence of this fusion was confirmed by reverse-transcription PCR followed by Sanger sequencing (Fig. 1g; Supplementary Information S1). Immunohistochemistry (IHC) for ROS1 was diffusely positive in the tumor cytoplasm (Fig. 1h). Fluorescence in situ hybridization (FISH) using a *ROS1* break-apart probe set showed the presence of an *ROS1* rearrangement with one fused (5'/3') signal and isolated 3' green signals (Fig. 1i; Supplementary Information S1).

### Oncogenic activity of *KLC1–ROS1* fusion

To investigate the oncogenic activity of the *KLC1–ROS1* fusion in glioma cells, we introduced the fusion into NIH/3T3 fibroblasts by lentiviral transduction and performed focus forming assays. Cells expressing the fusion demonstrated remarkable focus forming ability compared to control cells (Fig. 2a). Subsequently, to examine fusion-mediated oncogenic molecular signaling and phenotypes in glioblastoma cells, we generated two fusion mutants including an ROS1 kinase-dead mutant (KD) and a deletion mutant of the KLC1 portion (KLC1-del) of fusion (Fig. 2b). Fusion and mutant constructs were transiently introduced into A172 human glioblastoma cells, and lysates were analyzed by immunoblotting. The fusion, but not the ROS1

kinase-dead (KD) and *KLC1*-deletion (KLC1-del) mutants, induced prominent activation of JAK-STAT signaling, which is a downstream oncogenic pathway activated by ROS1, as shown by elevated JAK2 and STAT3 phosphorylation (Fig. 2c). In addition, A172 cells transiently expressing the fusion, but not the empty vector and the two mutants, exhibited increased cell proliferation and migration, which were suppressed by crizotinib, a clinically available ROS1 inhibitor (Fig. 2d). These results suggested that the *KLC1–ROS1* fusion is a transforming oncogene, and that both the KLC1 portion and ROS1 kinase activities are essential for its oncogenic activity.

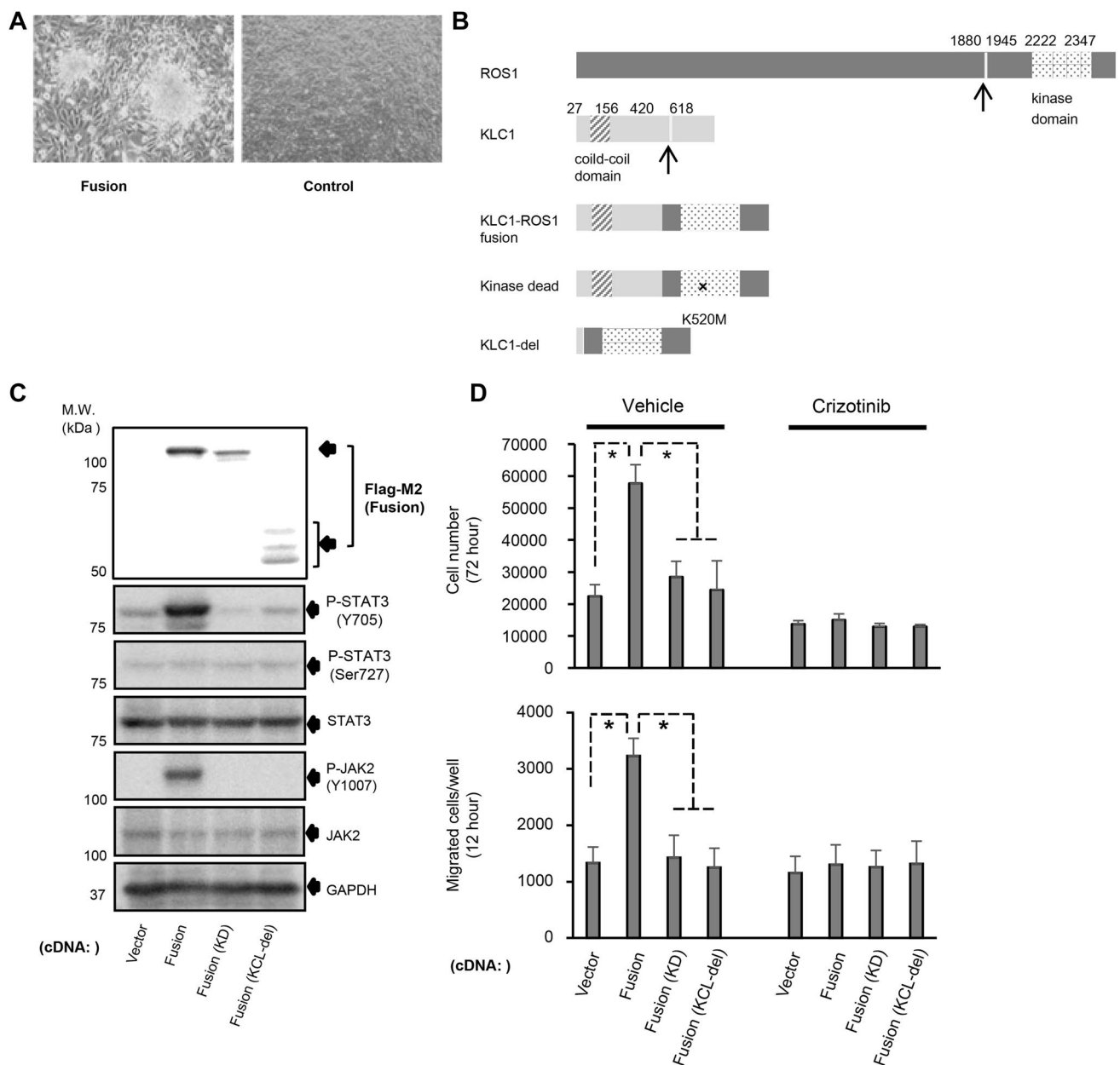
## Discussion

A novel *KLC1–ROS1* fusion was identified in a case of pediatric low-grade localized glioma; in vitro analysis demonstrated its oncogenic activity. No other pathogenic variants were detected based on the analysis of 93 genes associated with brain tumors. Hence, this novel fusion was likely a primary driver mutation in this case.

Kinesin light chain 1 (KLC1), encoded by the *KLC1* gene on chromosome 14, is involved in intracellular transport systems [4]. *KLC1–ALK* fusions have recurrently been detected in lung cancer, and the breakpoint of *KLC1* is the same as that found in the *KLC1–ROS1* fusion identified in this report, specifically, the 3' end of exon 9 of *KLC1* [5, 6]. Furthermore, the coiled-coil domain of KLC1 was retained in these fusions. The mechanism through which each fusion partner contributes to continuous activation of the kinase domain of ROS1 is not fully understood. However, more than half of previously reported ROS1 partners had a coiled-coil domain, and dimerization via these domains is thought to contribute to activation of the ROS1 kinase domain [2].

Interestingly, *ALK* or *ROS1* rearrangements have been recently reported in a subset of pediatric brain tumors (Table 1) [7–12]. These patients tended to be infants and younger children, although the number of the reported cases was small. Patients with these rearrangements might benefit from crizotinib or next-generation ALK/ROS1 inhibitors, which have shown promising activities against lung tumors that metastasize to the CNS [12, 13]. These targeted therapies might serve as an ideal alternative to the conventional chemotherapy and radiotherapy, which unfavorably affect the growth and development of children.

RNA sequencing is currently the most comprehensive and ideal method to detect fusion genes. However, it is time- and cost-consuming, and might not be routinely available in the clinical setting. Therefore, it is necessary to establish a practical approach to screen gene fusions. *ALK* or *ROS1* rearrangements can be detected by FISH. However, *GOPC–ROS1* fusions, which have been recurrently observed



**Fig. 2** Functional analysis of the *KLC1-ROS1* fusion. **a** NIH3T3 cells were infected with a lentiviral vector expressing the empty vector (control) or the wild-type *KLC1-ROS1* fusion, as indicated, and analyzed by focus forming assays. The magnified images of each group of treated cells obtained by phase-contrast microscopy are shown. **b** Schematic illustration of *KLC1-ROS1* mutants generated in this study. The number indicates amino acid (aa) position and the break-points are indicated by arrows. **c** Indicated *KLC1-ROS1* constructs were expressed in A172 glioma cells. After 72 h, the cell lysates were

analyzed by immunoblotting using indicated antibodies. A GAPDH antibody was used to control for protein loading. **d** (Upper panel) indicated *KLC1-ROS1* constructs were expressed in A172 glioma cells after treatment with vehicle (DMSO) or the ROS1 inhibitor crizotinib (500 nM). After 72 h, the number of cells in each treatment group was analyzed by proliferation assays.  $*p < 0.01$ . (Lower panel) indicated that *KLC1-ROS1* constructs were expressed in A172 glioma cells. After 72 h, the cells were analyzed by migration assays in the presence of vehicle (DMSO) or crizotinib (500 nM).  $*p < 0.01$

in pediatric glioma, can be difficult to detect by FISH analysis, because both genes are closely located on 6q22.1 and a specifically designed probe set is required for its detection [2, 14]. These facts highlight the importance of probe design. In fusion-positive cases, ALK or ROS1 protein expression

can be detected by IHC. We previously established this protocol using a large number of lung carcinomas whose ROS1 rearrangement status was available [15]. In addition, we previously used the same protocol to successfully identify ROS1-rearranged inflammatory myofibroblastic tumors [16,



**Table 1** Previously reported cases of pediatric brain tumor with ALK or ROS1 fusion

Previously reported cases of pediatric brain tumor with ALK or ROS1 fusion.									
Case no	Age	Sex	Histopathology	ALK or ROS1 fusion	Tumor location	Resection	Chemotherapy	Survival	References
1	0	M	Glioblastoma	<i>ZCCHC8-ROS1</i>	Hemispheric	None	None	Intra-operative death	Coccé et al. [8]
2	4	F	High-grade glioma	<i>GOPC-ROS1</i>	Thalamus, lateral ventricle	GTR	Chemotherapy, HDC with ASCR	Alive (> 2 year)	Kiehna et al. [11]
3	n/a	n/a	Low-grade glioma, NOS	<i>GOPC-ROS1</i>	n/a	n/a	n/a	n/a	Johnson et al. [7]
4	n/a	n/a	Diffuse astrocytoma	<i>CEP85L-ROS1</i>	n/a	n/a	n/a	n/a	Johnson et al. [7]
5	1	M	possible atypical meningioma or IMT	<i>TFG-ROS1</i>	Intraventricule	PR	Crizotinib	Alive (> 5 year)	Rossing et al. [12]
6	3	F	Low-grade glioma	<i>KLC1-ROS1</i>	Hemispheric	GTR	None	Alive (> 7 year)	This case
7	0	M	Ependymoma-like glioma	<i>KTNI-ALK</i>	Hemispheric	STR	None	Alive(> 6 year)	Olsen et al. [9]
8	0	F	Ependymoma-like glioma	<i>CCDC88A-ALK</i>	Hemispheric, leptomeningeal meta	GTR	Chemotherapy	Alive(> 3 year)	Olsen et al. [9]
9	0	F	High-grade glioma	<i>PPP1CB-ALK</i>	Hemispheric	GTR	None	Alive(> 3 year)	Aghajan [10]
10	n/a	n/a	High-grade glioma, NOS	<i>EML4-ALK</i>	n/a	n/a	n/a	n/a	Johnson et al. [7]

*n/a* no available data, *IMT* inflammatory tumor, *HDC* high-dose chemotherapy, *SCR* autologous stem-cell rescue, *GTR* gross total resection, *PR* partial resection, *STR* subtotal resection

[17]. With regard to the CNS tumors, one study reported that ROS1 IHC using the same antibody clone showed no ROS1 reactivity in 109 glioblastomas that lacked ROS1 fusions by FISH [3]. We, too, have not seen ROS1 IHC reactivity in more than 50 glioblastomas that lacked ROS1 rearrangement by FISH (unpublished data). Taken together, it is eminently possible that ROS1 IHC could serve as a screening tool or surrogate for ROS1-rearranged tumors in the CNS. However, future studies, including a large number of CNS tumors of different types, are necessary to test its utility.

Thus, it is recommended that both FISH and IHC be performed, followed by RNA sequencing when applicable.

In summary, a novel *KLC1-ROS1* fusion has been identified in a case of pediatric low-grade, localized glioma. Further investigations will help to determine any common features of brain tumors harboring ROS1 rearrangements and which patients should receive screening tests; moreover, the therapeutic benefit and appropriate use of ROS1 inhibitors for these patients should be validated in clinical trials.

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## Compliance with ethical standards

**Conflict of interest** The authors declare no conflicts of interest associated with this manuscript.

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