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3 **Spinal cord astroblastoma with *EWSR1-BEND2* fusion classified as HGNET-MN1**

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6 **by methylation classification: A case report**

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10
11
12 **Takeyoshi Tsutsui**

13
14
15
16 Department of Neurosurgery, Kyoto University Graduate School of Medicine, Kyoto,
17
18
19 606-8507, Japan

20
21
22 **Yoshiki Arakawa**

23
24
25 Department of Neurosurgery, Kyoto University Graduate School of Medicine, Kyoto,
26
27
28 606-8507, Japan

29
30
31 **Yasuhide Makino**

32
33
34 Department of Neurosurgery, Kyoto University Graduate School of Medicine, Kyoto,
35
36
37 606-8507, Japan

38
39
40
41 **Hiroharu Kataoka**

42
43
44 Department of Neurosurgery, Kyoto University Graduate School of Medicine, Kyoto,
45
46
47 606-8507, Japan

48
49
50
51 **Yohei Mineharu**

52
53
54 Department of Neurosurgery, Kyoto University Graduate School of Medicine, Kyoto,
55
56
57 606-8507, Japan

1
2
3 **Kentaro Naito**
4

5
6 Department of Neurosurgery, Osaka City University Graduate School of Medicine, Osaka
7
8
9 545-8585, Japan.
10

11
12 **Sachiko Minamiguchi**
13

14
15
16 Department of Diagnostic Pathology, Kyoto University Graduate School of Medicine,
17
18
19 Kyoto, 606-8507, Japan
20

21
22 **Takanori Hirose**
23

24
25 Department of Diagnostic Pathology, Hyogo Cancer Center, Akashi, Hyogo, 673-8558,
26
27
28 Japan
29

30
31 **Sumihito Nobusawa**
32

33
34
35 Department of Human Pathology, Gunma University Graduate School of Medicine,
36
37
38 Gunma, 371-8514, Japan
39

40
41 **Yoshiko Nakano**
42

43
44
45 Division of Brain Tumor Translational Research, National Cancer Center Research
46
47
48 Institute, Tokyo, 104-0045, Japan
49

50
51 **Koichi Ichimura**
52

53
54
55 Division of Brain Tumor Translational Research, National Cancer Center Research
56
57
58 Institute, Tokyo, 104-0045, Japan
59
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1
2
3 **Hironori Haga**
4

5
6 Department of Diagnostic Pathology, Kyoto University Graduate School of Medicine,
7
8
9 Kyoto, 606-8507, Japan
10

11
12 **Susumu Miyamoto**
13

14
15
16 Department of Neurosurgery, Kyoto University Graduate School of Medicine, Kyoto,
17
18
19 606-8507, Japan
20
21
22
23
24

25 **Corresponding author:**
26

27
28 Yoshiki Arakawa M.D., Ph.D.
29

30
31
32 Department of Neurosurgery, Kyoto University Graduate School of Medicine, 54
33
34
35 Kawahara-cho Shogoin Sakyo-ku, Kyoto 606-8507, Japan
36

37
38 TEL: +81-75-751-3459; FAX: +81-75-752-9501
39

40
41 E-mail: arakawa@kuhp.kyoto-u.ac.jp
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Abstract

The most recurrent fusion of central nervous system high-grade neuroepithelial tumor with *MNI* alteration (HGNET-*MNI*) is *MNI* rearrangement. Here, we report the case of a 36-year-old man with spinal cord astroblastoma showing *Ewing Sarcoma breakpoint region 1/EWS RNA-binding protein 1 (EWSR1)-BEN domain-containing 2 (BEND2)* fusion. The patient presented with back pain, gait disturbance and dysesthesia in the lower extremities and trunk. Magnetic resonance imaging showed an intramedullary tumor at the T3–5 level, displaying homogeneous gadolinium enhancement. Partial tumor removal was performed with laminectomy. Histological examinations demonstrated solid growth of epithelioid tumor cells showing high cellularity, a pseudopapillary structure, **intervening hyalinized fibrous stroma**, and some mitoses. Astroblastoma was diagnosed, classified as HGNET-*MNI* by the German Cancer Research Center methylation classifier. *MNI* alteration was not detected by fluorescence *in situ* hybridization (FISH), but *EWSR1-BEND2* fusion was detected by FISH and RNA sequencing. Previously, a child with *EWSR1-BEND2* fusion-positive spinal astroblastoma classified as HGNET-*MNI* was reported. In conjunction with that, the present case provides evidence that *EWSR1-BEND2* fusion is identified in the entity of HGNET-*MNI*. **Taken together, the *BEND2***

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3 alteration rather than *MNI* may determine the biology of a subset of the central nervous
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6 system HGNET-*MNI* subclass.
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10 11 12 **Keywords**

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14 astroblastoma, CNS high-grade neuroepithelial tumor with *MNI* alteration (HGNET-
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16 *MNI*), Ewing Sarcoma breakpoint region 1/EWS RNA-binding protein 1 (*EWSR1*), BEN
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18 domain-containing 2 (*BEND2*), *EWSR1-BEND2* fusion
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28 29 **Introduction**

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31 Astroblastomas are rare neuroepithelial tumors of unknown origin [8, 18],
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33 arising mostly in the cerebrum and rarely in the spinal cord. These tumors most often
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35 occur in the first to fourth decades of life, and show a female predominance [8, 18].
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38 Although their status as a pathological entity has not been fully determined, the genetic
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40 architecture of these tumors has gradually been elucidated.
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48 In 2016, genome-wide DNA methylation analyses of primitive neuroectodermal
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50 tumors of the central nervous system (CNS-PNETs) identified four new molecular entities,
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52 and one new entity termed CNS high-grade neuroepithelial tumor with *MNI* alteration
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54 (HGNET-*MNI*) was overrepresented by astroblastoma [13]. Similarly, around half of
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3 pathologically diagnosed astroblastomas were classified as CNS HGNET-*MNI* [7]. This
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6 group has been characterized by *MNI* (22q12.3) rearrangement, in which BEN domain-
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9 containing 2 (*BEND2*; Xp22.13) and CXXC-type zinc-finger protein 5 (*CXXC5*; 5q31.2)
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12 are the main fusion partners of the *MNI* gene [13]. In 2020, our research group reported
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15 the case of a 3-month-old boy with spinal astroblastoma, classified as CNS HGNET-*MNI*
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18 by the German Cancer Research Center (DKFZ) methylation classification [3, 17] but
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21 lacking *MNI* rearrangement. The tumor instead showed gene fusion between *Ewing*
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24 *sarcoma breakpoint region 1/EWS RNA-binding protein 1* (*EWSR1*) and *BEND2* [17].
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27 Herein, we report the case of another patient with primary spinal astroblastoma showing
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31 *EWSR1-BEND2* fusion.
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38 **Clinical summary**

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41 A 36-year-old man presenting with back pain, gait disturbance and dysesthesia
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44 in the lower extremities and trunk was referred to our institution. Neurological
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47 examination demonstrated bilateral dysesthesia below the T10 level and thermal
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50 hypoalgesia below the S2 level on the right, accompanied by motor weakness of the lower
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53 limbs that required a walking aid. Magnetic resonance imaging (MRI) showed a well-
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56 demarcated intramedullary tumor spreading exophytically at the T3–5 level, displaying
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3 hypointensity on T1-weighted imaging, hyperintensity on T2-weighted imaging, and
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6 homogeneous gadolinium enhancement (Fig. 1a-c). No lesion was observed in either the
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9 cerebrum or cerebellum. In addition, ^{18}F -fluorodeoxyglucose-positron emission
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12 tomography showed no apparent accumulation in the tumor (data not shown). The patient
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15 underwent partial tumor removal via T3–5 laminectomy. The tumor formed a solid extra-
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18 axial mass, which looked grayish (Fig. 1d). The exophytic portion was well-demarcated
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21 and able to be safely peeled off the spinal cord. The intramedullary portion displayed no
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25 clear boundary.
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29 Postoperatively, the residual tumor showed rapid growth with necrotic change.
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32 Based on the previous report showing that radiotherapy with concomitant chemotherapy
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35 was effective, the patient underwent focal irradiation with 54 Gy in 30 fractions along
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38 with bevacizumab (10 mg/kg) and temozolomide (75 mg/m^2). In the late phase of the
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41 adjuvant treatment, the tumor reduced in size and perifocal edema was also diminished
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44 (Fig. 1. e-h). The patient recovered from back pain and started to walk without assistance.
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48 However, massive dissemination of the tumor was identified 12 months after
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51 chemoradiotherapy (Fig. 1i).
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57 **Pathological findings**

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Histopathological examination of the tumor tissue showed solid growth of epithelioid tumor cells. A vague perivascular arrangement of tumor cells (Fig. 2a), those in a pseudopapillary pattern (Fig. 2b), and intervening hyalinized fibrous stroma (Fig. 2c) were occasionally observed, but astroblastic pseudorosettes were not discernible. The tumor cells possessed round-to-oval nuclei and relatively abundant eosinophilic cytoplasm with 4 mitoses per 10 high-power fields (Fig. 2d). No necrosis, true rosettes, or calcification was seen. Immunohistochemical analyses showed that tumor cells were strongly and diffusely positive for epithelial membrane antigen (EMA) (Fig. 2e) and focally positive for glial fibrillary acidic protein (GFAP) (Fig. 2f), with nuclear positivity for Olig2 (Fig. 2g) and Ki-67 labeling index 20% (Fig. 2h). Tumor cells were also positive for S-100, but negative for p53, progesterone receptor, and cytokeratin AE1/AE3 (data not shown). A histological diagnosis of astroblastoma was established.

The tumor was classified as HGNET-*MNI* according to the DKFZ methylation classifier[3], with a calibrated score of 0.98. We then analyzed *MNI* and *BEND2* rearrangements by fluorescence *in situ* hybridization (FISH). No *MNI* rearrangement was detected (Fig. 3a). Due to the previous case of a child showing *EWSR1-BEND2* fusion in astroblastoma, we performed FISH analysis using *EWSR1* and *BEND2* probes. *EWSR1* rearrangement as well as colocalization of *EWSR1* and *BEND2* probes was detected (Fig.

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3 3b, c). Consistent with RNA sequencing and following FISH analysis suggesting
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6 *EWSR1-BEND2* fusion, direct sequencing of the cDNA indicated that the breakpoints
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9 fell within exon 7 in *EWSR1* and exon 2 in *BEND2*, resulting in an in-frame fusion (Fig.
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13 3d).

14 15 16 17 18 19 **Discussion**

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22 Astroblastoma is a rare and diagnostically challenging glial tumor, accounting
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25 for 0.48–2.8% of all gliomas [1]. Astroblastoma occurs mainly in children and young
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28 adults. Most astroblastomas occur in the cerebral hemispheres, with the frontal lobe as
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31 the most common primary site [12]. Although brainstem and spinal cord origins have
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34 been reported on rare occasions [12, 16, 17], most such cases have been reported recently,
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37 probably due to advances in the genetic classification of CNS tumors. The majority of
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40 histologically diagnosed astroblastomas have been classified as CNS HGNET-*MNI*, with
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43 the remaining classified as pleomorphic xanthoastrocytoma, glioma or ependymoma [14,
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47 15]. Pediatric cerebral astroblastomas that were classified as CNS HGNET-*MNI* were
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50 reported to show better prognosis, although the significance of the genetic signature in
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53 spinal HGNET-*MNI* remains undetermined [14].

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57 The first case of primary spinal astroblastoma was reported only recently, by
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3 Yamada et al. in 2018 [16]. This may stem from reporting bias: astroblastoma is not
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6 usually recognized as a differential diagnosis for spinal cord tumor, and some cases might
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9 be misclassified as ependymoma or astrocytoma. Thanks to advances in genetic analyses
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12 such as methylation classifier [3], a final diagnosis has become easier to reach with more
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15 confidence. Although histologically diagnosed astroblastoma and HGNET-*MNI* may not
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18 be identical, molecular classification of HGNET-*MNI* by methylation analysis was
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21 compatible with the diagnosis of astroblastoma in the present case. Three cases of spinal
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24 astroblastoma have been reported to date, including our own. These three cases are
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27 summarized in Table 1 [16, 17]. The tumor was located in the upper cervical spine in one
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30 pediatric case and in the upper thoracic spine for the other two tumors in young adults.
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34 All three tumors showed an invasive growth pattern despite a well-defined border on MRI
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37 examination, and were all treated by partial resection followed by chemoradiotherapy.
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41 This treatment showed efficacy in both young adult cases, although the pediatric case
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44 proved resistant to the treatment and showed dismal prognosis. The first case was
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47 suggested to show a chromosomal structural abnormality involving the *MNI* gene by
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50 FISH. Interestingly, EWSR1-BEND2 fusion without *MNI* rearrangement was detected in
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53 two cases, with methylation patterns compatible with CNS HGNET-*MNI* according to
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56 the DKFZ classifier. Whether this type of fusion is specific to spinal astroblastoma
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3 warrants further investigation. Spinal astroblastoma is an extremely rare tumor, but
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6 ependymoma- or anaplastic ependymoma-like tumors or other spinal glial tumors in the
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9 spinal region with some unusual findings may be reclassified into astroblastoma if tested
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12 by genetic analysis. Thus, previous cases of spinal tumor need to be reviewed, and
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15 astroblastoma should be considered among the differential diagnoses for spinal tumors,
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18 especially in cases involving children and young adults.
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25 HGNET-*MNI* demonstrates variable morphological and immune-phenotypical
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28 features. The characteristic features are astroblastic pseudorosettes and stromal sclerosis
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31 [14]. In the present case, only the **hyalinized fibrous stroma** was identified, while
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34 astroblastic pseudorosettes were not well observed [6]. Immunoreactivities for GFAP, S-
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37 100 protein, Olig2, and EMA were variably demonstrated in HGNET-*MNI* [5, 14].
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41 Only four cases with *EWSR1-BEND2* fusion have been previously reported: one
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44 pancreatic neuroendocrine tumor [11], one spinal ependymoma [9], one spinal
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47 astroblastoma [17] and the present case. Three of four *EWSR1-BEND2* fusion tumors
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50 were localized in the spinal cord. Fusion between *EWSR1* (22q12.2) and partners, i.e.,
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53 erythroblastosis virus-transforming sequence (avian ETS) transcription factor family,
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56 causes various mesenchymal tumors, including Ewing's sarcoma [10]. The N-terminal
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3 transcriptional activating domain of *EWSR1* contributes to tumorigenesis. *BEND2* protein
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6 is characterized by two BEN domains that bind DNA and are involved in transcription
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9 and chromatin regulation [4]. Both domains derived from *EWSR1* and *BEND2* are
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12 necessary for tumorigenesis [17]. In addition, in both cases of fusion with *MN1* [2] and
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15 *EWSR1* [17], RNA sequencing revealed increased expression of *BEND2* downstream
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18 from the breakpoints [13]. This indicates that the fusion of *BEND2* enhances transcription
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21 of *BEND2*, which might be associated with oncogenesis. As suggested by a previous
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24 report, the presence of *EWSR1-BEND2* fusion tumors could indicate that *BEND2* rather
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27 than *MN1* may define the biology of a subset of CNS HGNET [17]. Accumulation of case
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30 series as well as functional analyses of fusion genes are needed to clarify the
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33 pathophysiology of *EWSR1-BEND2*-positive astroblastoma and CNS HGNET-*MN1*.
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41 **List of abbreviations**

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44 HGNET-MN1: CNS high-grade neuroepithelial tumor with MN1 alteration; EWSR1:
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47 Ewing Sarcoma breakpoint region 1/EWS RNA-binding protein 1; BEND2: BEN
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50 domain-containing 2; DKFZ: the German Cancer Research Center; FISH: fluorescence
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53 *in situ* hybridization; CNS-PNETs: primitive neuroectodermal tumors of the central
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7 **Declarations**
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10 **Ethics Statement and Consent for publication**
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12 This report was carried out in accordance with the principles of the Declaration of
13 Helsinki, and approval was obtained from the institutional review boards at Kyoto
14 University Hospital (approval number: R1285, R2088). Informed consent was obtained
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22 from the patient for publication.
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29 **Availability of data and material**
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31 Not applicable.
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39 **Competing interests**
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41 The authors declare that they have no competing interests.
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Authors' contributions

T.T. and Y.A. designed the study of the tumor. S.M., T.H., and H.H. performed histological analyses and diagnosis. S.N., Y.N., and K.I. performed genetic analyses and data analyses. Y.M., H.K., Y.M., K.N., and S.M. collected samples. T.T. and Y.A. wrote the manuscript. All authors read and approved the final manuscript.

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39 features: a cytological, histological, and immunohistochemical study. Brain

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46 Tumor Pathol 33: 63-70 Doi 10.1007/s10014-015-0241-5

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48 **Table 1.** Summary of cases of spinal astroblastoma

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51 Postoperative

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53 Author	54 Age	55 Sex	56 Location	57 Ki-67	58 Genetic analysis	59 Treatment	60 survival
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3	Yamada et al.,							1 year
4		20 years	female	T1	5%	<i>MNI</i> rearrangement	RT+TMZ+BEV	
5								
6	2018							(alive)
7								
8								
9	Yamasaki et al,					<i>EWSR1-BEND2</i>	RT+TMZ+ETP /	30 days
10		3 months	male	medulla-C4	34%			
11								
12	2020					fusion	BEV	(dead)
13								
14								
15								
16						<i>EWSR1-BEND2</i>		2 years
17	Present case	36 years	male	T3-5	20%		RT+TMZ+BEV	
18								
19						fusion		(alive)
20								

RT: radiation therapy, TMZ: temozolomide, BEV: bevacizumab, ETP: etoposide.

Figure Legends

Figure 1. Sequential MRI at initial diagnosis and after partial tumor resection

a–c: An intra-axial tumor is identified at the T3–5 level. T2-weighted imaging demonstrates peritumoral edema in the spinal cord (a). The tumor shows homogeneous enhancement (b, c). d: Intraoperative view of the tumor. An exophytic tumor is observed at the T4 level, showing grayish coloration. e–f: At 1 month postoperatively, residual tumor at the T4 level shows rapid growth and perifocal edema extending downward to the C5 level (e). Gadolinium-enhanced T1-weighted imaging shows necrotic changes at the center of the tumor (f). g–h: In late phase of radiotherapy concomitant with

1
2
3 chemotherapy using temozolomide and bevacizumab, perifocal edema resolves (g) in
4
5
6 association with a reduction in tumor size (h). Massive dissemination of the tumor was
7
8
9 identified 12 months after chemoradiotherapy (i).

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16 **Figure 2.** Histopathological examination of the resected spinal tumor

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18 a–c: Low magnification ($\times 10$) images with hematoxylin and eosin (HE) staining show
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a–c: Low magnification ($\times 10$) images with hematoxylin and eosin (HE) staining show
perivascular arrangement of tumor cells (a) and a pseudopapillary pattern (b). A higher
magnification ($\times 40$) images show intervening hyalinized fibrous stroma (c), epithelial-
like tumor cells with round-to-oval nuclei and eosinophilic cytoplasm and a mitotic figure
(white arrow) (d). e–h: Immunohistochemically, tumor cells display strong, diffusely
positive staining for EMA (e), focally positive staining for GFAP (f), and positive nuclear
staining for Olig2 (g). Ki-67 labeling index is 20% (h).

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Figure 3. Genetic analyses of the resected spinal tumor

a–c: FISH analysis. No *MNI* rearrangement is identified (a: red, *MNI* centromeric probe;
green, *MNI* telomeric probe). *EWSR1* rearrangement is detected as a split signal with
break-apart of two probes for *EWSR1* (b: arrows; red fluorescence, *EWSR1* centromeric
probe; green, *EWSR1* telomeric probe). *EWSR1-BEND2* fusion is suggested by

1
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3 colocalization of the red *EWSR1* centromeric probe and the green *BEND2* probe (c: red
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5
6 fluorescence, *EWSR1* centromeric probe; green, encompassing the *BEND2* gene). d:
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9 Direct sequence of the cDNA at the break point shows fusion between *EWSR1* exon 7 and
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13 *BEND2* exon 2.
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Figure 1

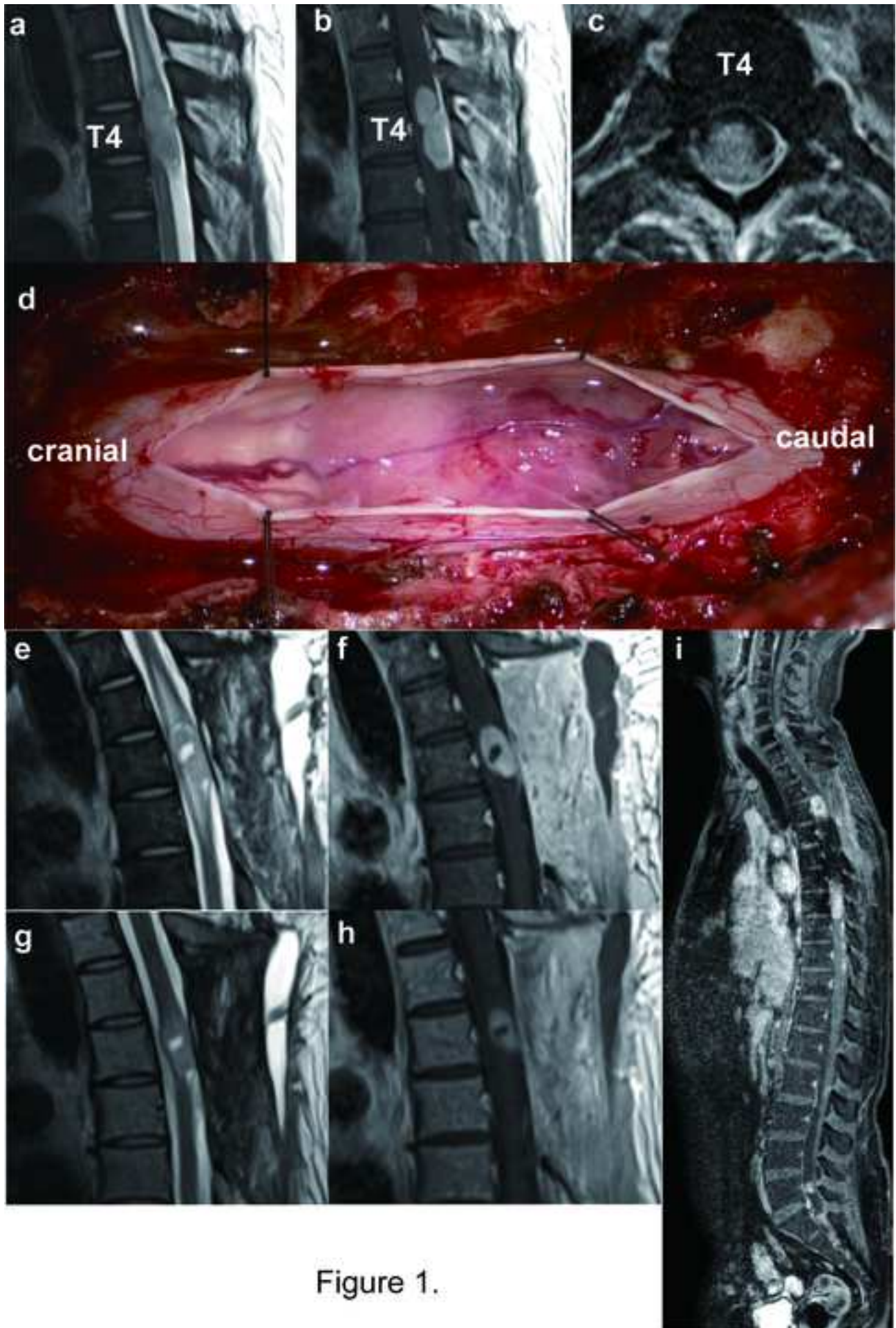


Figure 1.

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Figure 2

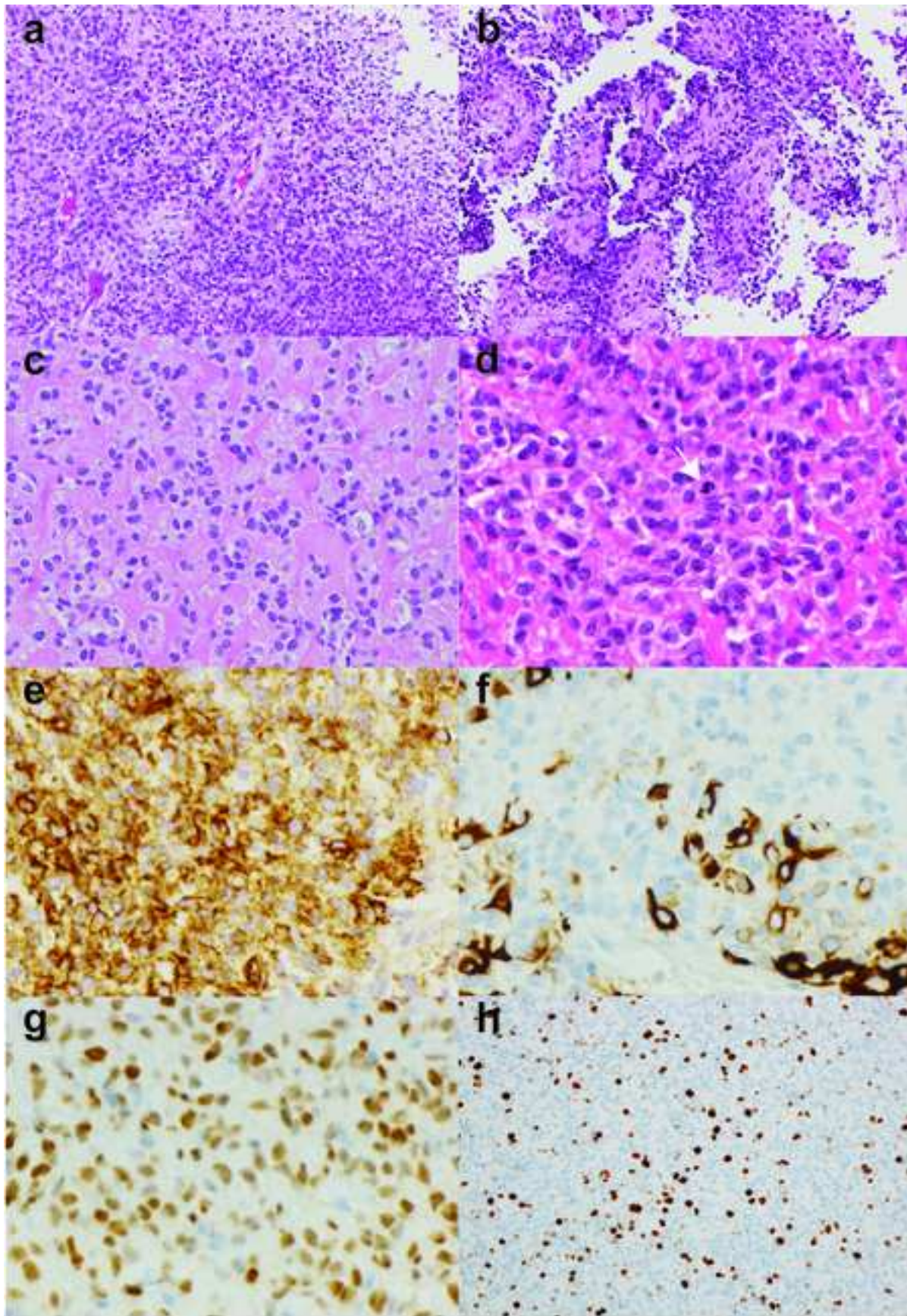


Figure 2.

