

# Intracranial Mesenchymal Chondrosarcoma Lacking the Typical Histopathological Features Diagnosed by *HEY1-NCOA2* Gene Fusion

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Intracranial mesenchymal chondrosarcoma (MCS) is a rare neoplasm. The diagnosis of MCS is confirmed by the presence of a biphasic pattern on histological examination, comprising undifferentiated small round cells admixed with islands of well-differentiated hyaline cartilage; however, a differential diagnosis may be challenging in some cases. A 28-year-old woman with a 2-month history of headache was referred to our hospital. Radiologic studies showed an extra-axial lobulated mass composed of calcified and uncalcified areas occupying the left middle fossa. Surgical resection was planned, but her headache suddenly worsened before her planned hospital admission and she was admitted as an emergency. Radiologic studies showed an acute hemorrhage in the uncalcified part of the mass. The mass was resected via the left zygomatic approach after embolization of the feeder vessels. The most likely histopathological diagnosis was MCS. However, the typical bimorphic pattern was not identified in our surgical samples; each undifferentiated area and well-differentiated area was observed separately in different tissue specimens, and no islands of well-differentiated hyaline cartilage were identified within the undifferentiated areas in the same specimen. Molecular assays confirmed the presence of *HEY1-NCOA2* fusion. *IRF2BP2-CDX1* fusion and *IDH1/2* mutations were negative. The final diagnosis of MCS was made based on the presence of *HEY1-NCOA2* gene fusion. MCS should be included in the differential diagnosis when radiologic studies show an extra-axial lobulated mass with calcification. Furthermore, molecular demonstration of *HEY1-NCOA2* gene fusion may help make a precise diagnosis of MCS, especially in surgical samples lacking the typical histopathological features.

**Keywords:** gene fusion, *HEY1-NCOA2*, *IRF2BP2-CDX1*, mesenchymal chondrosarcoma

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## Introduction

Intracranial chondrosarcoma (CS) is a rare neoplasm representing only 6% of all skull-base tumors and 0.1–0.15% of all intracranial tumors, with an annual incidence of <0.02/100,000.<sup>1–4</sup> Moreover, mesenchymal CS (MCS), as the most malignant subtype of CS with the poorest prognosis, represents only 2–10% of all CS cases and 16–20% of intracranial CS cases.<sup>4,5</sup>

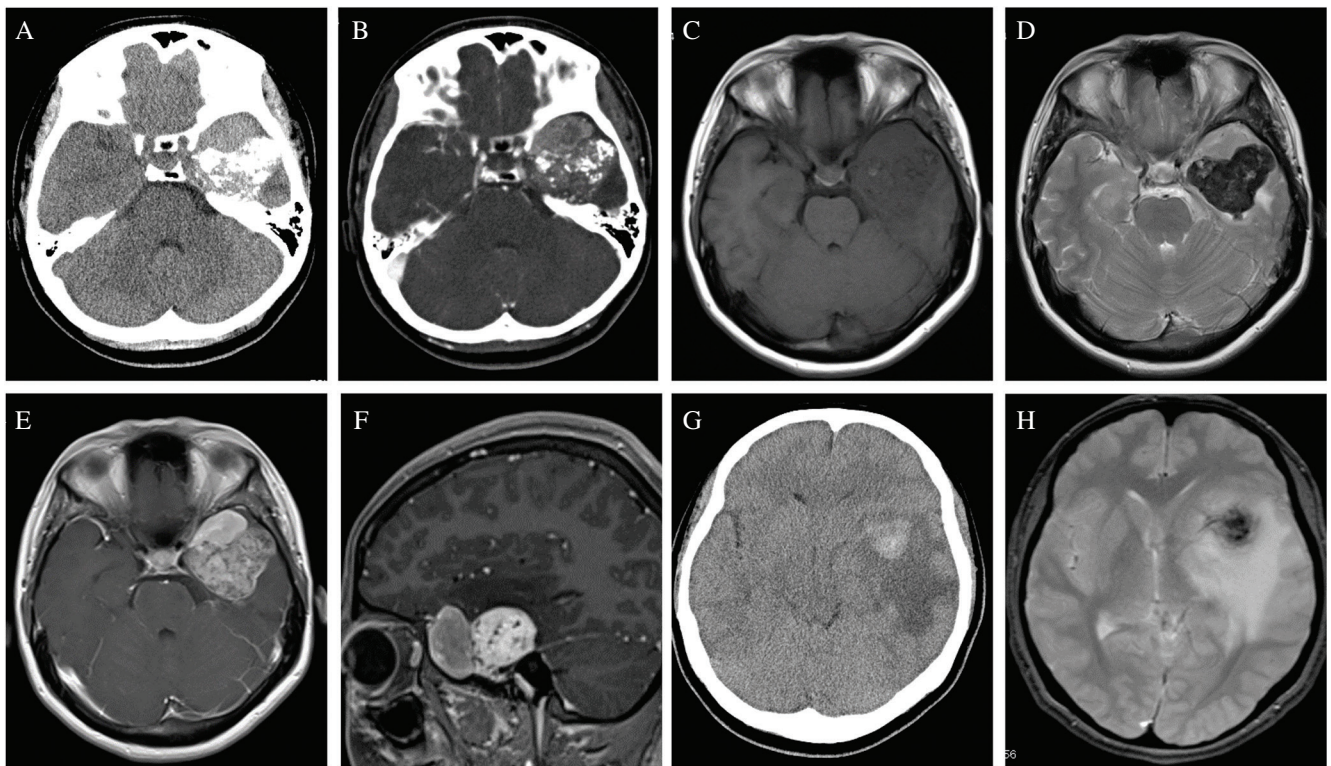
Two novel specific fusion genes, *HEY1-NCOA2* and *IRF2BP2-CDX1*, have been identified in MCS, based on genome-wide analysis.<sup>6–8</sup> The diagnosis of MCS is relatively apparent when a classic bimorphic pattern comprising undifferentiated small round to spindle-shaped cells admixed with islands of well-differentiated hyaline cartilage is detected by histological analysis.<sup>9,10</sup> However, a fairly broad differential diagnosis may be required in some cases, such as an unusual clinical presentation, insufficient material, and/or lack of apparent histological bimorphism.<sup>10–12</sup> Although immunohistochemistry may help make a definitive diagnosis, specific immunohistochemical markers for MCS are currently limited.<sup>13,14</sup> Molecular demonstration of the specific fusion genes, *HEY1-NCOA2* and *IRF2BP2-CDX1*, may thus help or even be required to differentiate these tumors in diagnostically challenging cases.<sup>7,8,12,15,16</sup>

In this report, we describe a rare case of intracranial MCS diagnosed by *HEY1-NCOA2* gene fusion and highlight the usefulness of molecular assays in surgical samples lacking the typical histopathological features.

## Case Report

### History and presentation

A 28-year-old woman with a 2-month history of headaches was referred to our hospital because of a mass occupying the left middle fossa. Head computed tomography (CT) showed a 5.0-cm diameter left hyperdense extra-axial lobular mass with heterogeneous contrast enhancement and peritumoral edema (Figs. 1A and 1B). The mass had a two-component structure composed of calcified and uncalcified areas. The posterior part of the mass showed significant calcification. Head magnetic resonance imaging (MRI) showed that the anterior uncalcified part of the lesion was iso-intense on T1- and T2-weighted images and the posterior calcified part of the lesion was iso-intense on T1-weighted images and hypo-intense on T2-weighted images (Figs. 1C and 1D). Both anterior and posterior parts of the lesion were heterogeneously enhanced on gadolinium contrast-enhanced

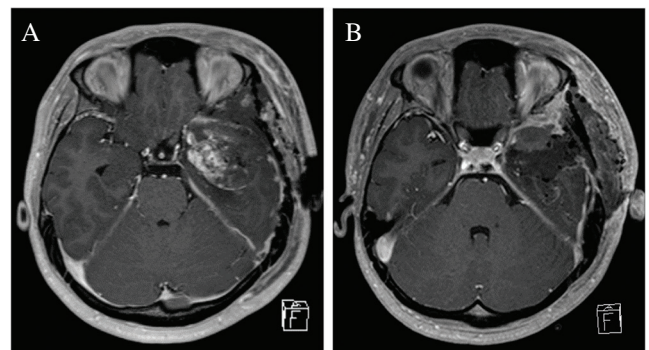


**Fig. 1** (A) Head computed tomography (CT) image showing an extra-axial lobular mass with calcification occupying the left middle fossa. (B) Contrast-enhanced head CT image showing heterogeneous enhancement of the mass. (C) T1-weighted head magnetic resonance (MR) image showing the iso-intense mass. (D) T2-weighted head MR image showing iso-intense anterior uncalcified part of the lesion and hypo-intense posterior calcified part of the lesion. (E and F) Axial and sagittal gadolinium contrast-enhanced T1-weighted MR images showing heterogeneous enhancement of the mass. (G and H) Head CT and T2-weighted MR images showing an acute hemorrhage in the anterior uncalcified part of the mass and midline shift.

T1-weighted images (Figs. 1E and 1F). The imaging-based differential diagnoses were meningioma, osteosarcoma, CS, or solitary fibrous tumor/hemangiopericytoma. Pre-surgical cerebral angiography and surgical resection was planned. However, her headache suddenly worsened with severe vomiting before her planned hospital admission, and she was admitted to our hospital as an emergency. Head CT and T2-weighted MRI showed an acute hemorrhage in the anterior uncalcified part of the mass and midline shift (Figs. 1G and 1H). Sub-emergent angiography was performed. Left external carotid angiography showed a blood supply from the petrosal branch of the left middle meningeal artery and artery of the foramen rotundum from the left internal maxillary artery. Left internal carotid angiography showed a blood supply from the recurrent meningeal branches of the left ophthalmic artery. Embolization of the feeder vessels from the external carotid artery was performed.

*Operation and postoperative course*

The day after embolization, the patient underwent sub-emergent resection of the mass via the left zygomatic approach to achieve decompression. The tumor was attached to the sphenoidal bone. The hemorrhagic part of the tumor was soft and was almost totally resected, achieving decompression (Fig. 2A). In contrast, the calcified part of the tumor was hard; therefore, we could not proceed and discontinued



**Fig. 2** (A) Post-first operation gadolinium contrast-enhanced T1-weighted magnetic resonance (MR) image showing almost total resection of the hemorrhagic anterior part of the tumor and partial resection of the calcified posterior part of the tumor. (B) Post-second operation gadolinium contrast-enhanced T1-weighted MR image showing almost total resection of the calcified posterior part of the tumor, except for the attachment part on the sphenoidal bone.

the surgery with the calcified section only partially resected (Fig. 2A). The postoperative course was uneventful. If the histological diagnosis had indicated benign tumors, we would have considered follow-up without additional resection for the remnant calcified tumor. However, a final diagnosis of MCS was made based on histological and molecular analyses as described below. Therefore, we performed a



second surgery. The remnant calcified tumor was softer than at the first surgery and was almost totally resected, except for the part attaching on the sphenoidal bone (Fig. 2B). The second postoperative course was also uneventful. At 20 days after her second surgery, the patient received adjuvant proton-beam therapy with a total dose of 70.4 Gy (2.2 Gy/fraction) for the residual tumor on the sphenoidal bone. A brain MRI at 6 months after surgery showed no evidence of recurrence.

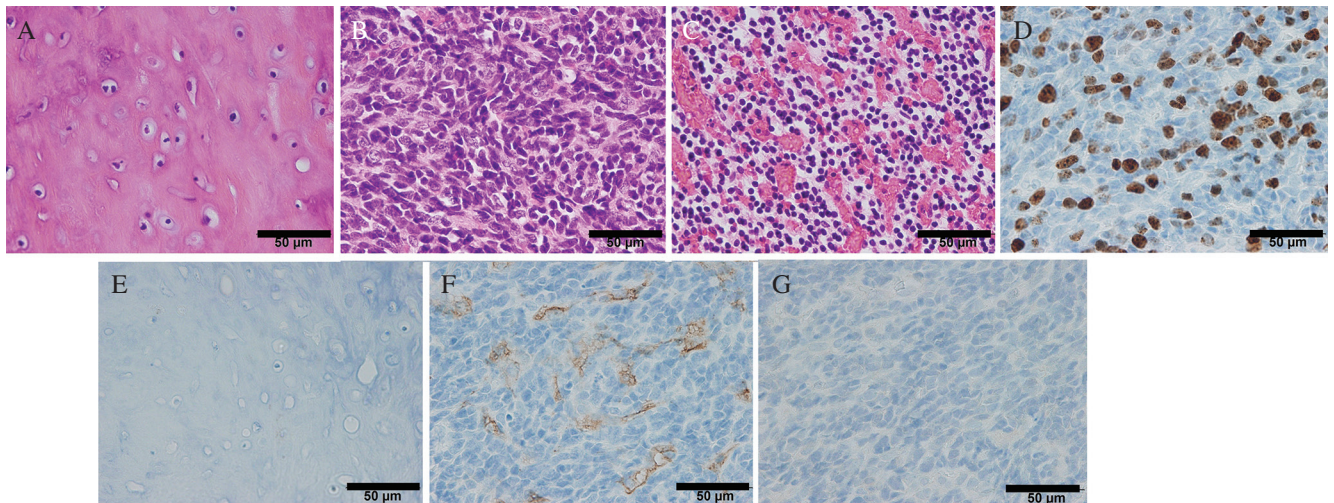
### Histological findings

Microscopic histopathological examination of the tumor specimen showed undifferentiated round or spindle-shaped cells with hemorrhage and well-differentiated cartilaginous/osseous components (hematoxylin and eosin) (Figs. 3A–3C). The Ki-67 labeling index was approximately 20% (Fig. 3D). Immunohistochemistry for S-100, CD34, and STAT6 was negative (Figs. 3E–3G). A solitary fibrous tumor/hemangiopericytoma was therefore excluded. The most likely histopathological diagnosis was MCS. However, the typical bimorphic pattern was not identified in our surgical samples; each undifferentiated area and well-differentiated area was observed separately in different tissue specimens, and islands of well-differentiated hyaline cartilage were not identified within the undifferentiated areas in the same tissue specimen.

### Molecular findings

We performed molecular assays to confirm the diagnosis of MCS. For detection of the *IDH1* and *IDH2* mutations, genomic DNA was extracted from fresh-frozen tumor samples using a QIAamp DNA Micro Kit (Qiagen, Hilden, Germany) and amplified by polymerase chain reaction (PCR) using two primer sets for exon 4 of the *IDH1* gene and exon 4 of the *IDH2* gene, respectively. The primer sequences used are listed in Table 1. The PCR products were then sequenced on a 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) with a Big Dye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems), in accordance with the manufacturer's instructions.

For detection of the *HEY1-NCOA2* and *IRF2BP2-CDX1* fusion genes, total RNA was extracted from fresh-frozen tumor samples using a TRIzol Reagent Kit (Invitrogen, Life Technologies, Grand Island, NY, USA), and used to synthesize cDNA with a PrimeScript RT Reagent Kit (Takara Bio Inc., Shiga, Japan). The primer sequences used are listed in Table 1.<sup>7,16</sup> A cDNA synthesized from human brain total RNA (Invitrogen, Life Technologies) with a PrimeScript RT Reagent Kit (Takara Bio Inc.) was used as a negative control and the housekeeping gene *GAPDH* was amplified to check the cDNA quality in both samples, using the primers



**Fig. 3** (A) Well-differentiated cartilaginous/osseous components, (B) undifferentiated round or spindle-shaped cells, and (C) undifferentiated round cells with hemorrhage (all hematoxylin and eosin stain). (D) Ki-67 labeling index was approximately 20%. (E) Negative S-100 immunohistochemistry in the cartilage portion, (F) negative CD34 immunohistochemistry in the undifferentiated portion, and (G) negative STAT6 immunohistochemistry in the undifferentiated portion.

**Table 1** Primers used for molecular assays

Gene	Forward primer	Reverse primer
<i>IDH1</i>	GACCAAGTCACCAAGGATGC	TGTGTTGAGATGGACGCCTA
<i>IDH2</i>	GGGGTTCAAATTCTGGTTGA	CTCGTCGGTGTGTACATGC
<i>GAPDH</i>	GACCTGCCGTCTAGAAAAACC	GCTGTAGCCAAATTCGTTGTC
<i>HEY1-NCOA2</i> <sup>16)</sup>	CGAGATCCTGCAGATGACCGTGG	GCACCAGTTGGGCTTTGCAATGTG
<i>IRF2BP2-CDX1</i> <sup>7)</sup>	CAAGAGCCGCGGGTCTGGAGA	TGATGTCGTGGGCCATCGGC

listed in Table 1. The PCR products were then sequenced using a 3130xl Genetic Analyzer (Applied Biosystems) and a Big Dye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems), in accordance with the manufacturer’s instructions.

Both *IDH1* and *IDH2* mutations were negative (Figs. 4A and 4B). Agarose gel electrophoresis of the reverse transcription PCR products showed a single band of the expected length for the *HEY1-NCOA2* fusion product, but no band corresponding to the *IRF2BP2-CDX1* fusion product in the MCS sample (Fig. 4C). Sanger sequencing confirmed the presence of a transcript caused by fusion between exon 4 of *HEY1* and exon 13 of *NCOA2* (Fig. 4D). The final diagnosis of MCS was confirmed.

**Discussion**

We report a case of intracranial MCS diagnosed by detection of *HEY1-NCOA2* gene fusion. Our surgical samples lacked the typical bimorphic pattern comprising undifferentiated cells admixed with islands of well-differentiated hyaline cartilage; each area of undifferentiated cells and well-differentiated hyaline cartilage was observed separately in different tissue specimens. *HEY1-NCOA2* gene fusion was useful to confirm the diagnosis of MCS in our patient.

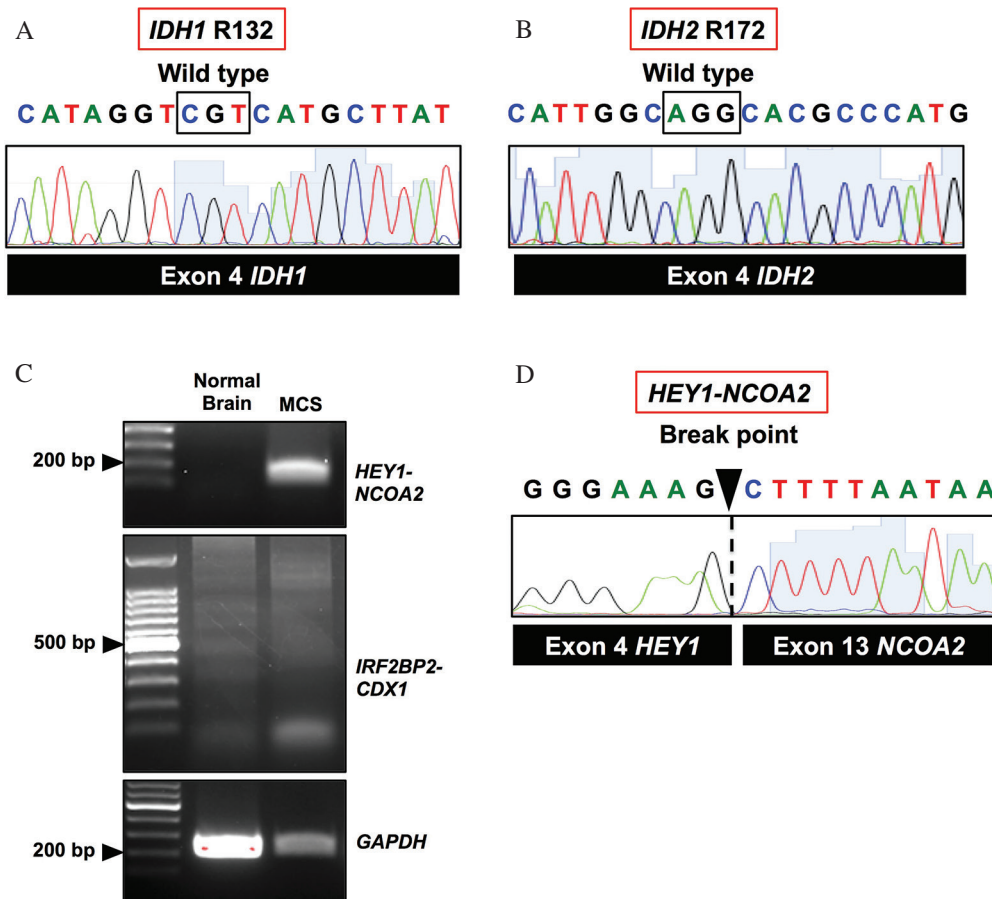
*Diagnosis of MCS by histological analysis*

Mesenchymal chondrosarcoma diagnosis has traditionally depended on the identification of diagnostic histologic

features, characterized by a biphasic pattern comprising undifferentiated small round to spindle-shaped cells admixed with islands of well-differentiated hyaline cartilage.<sup>9</sup> However, the diagnosis can be challenging if limited biopsy tissue is available or if the biphasic pattern is absent or subtle, with a predominance of only one of the two components.<sup>11</sup> Such cases warrant a broad differential diagnosis including meningioma, solitary fibrous tumor/hemangiopericytoma, oligodendroglioma, chordoma, poorly differentiated synovial sarcoma, Ewing sarcoma, lymphoma, small cell osteosarcoma, embryonal rhabdomyosarcoma, and other types of CS.<sup>16,17</sup>

Although immunohistochemistry may help make a definitive diagnosis, specific immunohistochemical markers for MCS are currently limited. SOX9 is useful as a specific immunohistochemical marker because MCS can show strong nuclear reactivity for SOX9, indicative of a chondroid lineage.<sup>13</sup> However, CD99 and S-100 are relatively nonspecific and can be positive in several other types of tumors included in the differential diagnoses.<sup>13</sup> Desmin and epithelial membrane antigen may also be aberrantly expressed in some cases, but smooth muscle actin, myogenin, MyoD1, glial fibrillary acidic protein, and keratins are negative.<sup>14</sup>

In our surgical samples, areas of undifferentiated small round cells and well-differentiated hyaline cartilage were both observed, but each area was observed separately in different tissue specimens, and no islands of well-differentiated hyaline cartilage were identified within the undifferentiated



**Fig. 4** (A) Tumor was negative for *IDH1* (R132) and (B) *IDH2* (R172) mutations. (C) Agarose gel electrophoresis of *HEY1-NCOA2* and *IRF2BP2-CDX1* fusion PCR products. A *HEY1-NCOA2* fusion polymerase chain reaction (PCR) product but no *IRF2BP2-CDX1* fusion PCR product was detected. DNA quality was checked with reference to *GAPDH*. (D) Partial sequence of the *HEY1-NCOA2* fusion transcript. The position of the breakpoint is indicated. bp: base pair, MCS: mesenchymal chondrosarcoma.

areas in the same tissue specimen. We therefore performed molecular assays to confirm the diagnosis of MCS.

#### Usefulness of molecular assays for diagnosing MCS

The introduction of a simple method for detecting specific fusion genes, such as *HEY1-NCOA2*, may be helpful or even necessary to confirm a diagnosis of MCS, especially in an atypical clinical and histological context.<sup>8,11,12)</sup>

A recurrent *HEY1-NCOA2* fusion has been identified in >80% of MCS cases.<sup>6)</sup> The chimeric fusion is generated from an intra-chromosomal deletion between exon 4 of *HEY1* (8q21) and exon 13 of *NCOA2* (8q13).<sup>6)</sup> This fusion is specific for MCS, and has not been found in other types of CS, such as dedifferentiated or conventional CS.<sup>6,7)</sup>

The *IRF2BP2-CDX1* fusion has also been identified in MCS, and was reported in a *HEY1-NCOA2*-negative case of MCS.<sup>7)</sup> This fusion is generated by a translocation between exon 1 of the *IRF2BP2* gene on chromosome 1 and intron 1 of the *CDX1* gene on chromosome 5.<sup>7)</sup>

Somatic mutations in *IDH1* and *IDH2* commonly occur in low-grade gliomas and acute myeloid leukemia.<sup>18,19)</sup> *IDH1/2* mutations are also found in conventional central and dedifferentiated CS, but not in peripheral CS and osteochondromas.<sup>20)</sup> The predominant type is *IDH1* R132C, which is uncommon in low-grade gliomas and acute myeloid leukemia.<sup>20)</sup> However, whether or not *IDH1/2* mutations co-exist with the *HEY1-NCOA2* fusion in MCS remains unknown.<sup>6)</sup> Furthermore, *IDH1/2* mutations were not identified in chordomas of the skull base, suggesting that *IDH1/2* mutation status could be valuable for distinguishing between intracranial CS and chordomas.<sup>17)</sup>

The current case showed *HEY1-NCOA2* but not *IRF2BP2-CDX1* gene fusion. These results are in accord with previous reports in which *IRF2BP2-CDX1* was absent in *HEY1-NCOA2*-positive cases of MCS.<sup>7)</sup> Furthermore, the *HEY1-NCOA2* fusion did not co-exist with *IDH1/2* mutations in the present case.

#### Conclusion

In conclusion, we report a case of intracranial MCS diagnosed by *HEY1-NCOA2* gene fusion. *IRF2BP2-CDX1* fusion and *IDH1/2* mutations were negative. Although MCS is extremely rare, it should be included in the differential diagnosis when radiologic studies show an extra-axial lobular mass with calcification. Molecular techniques are useful for detecting the presence of specific fusion genes and confirming a diagnosis of MCS, especially in surgical samples lacking the typical histopathological features.

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#### Ethical Approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. For this type of study formal consent is not required.

#### Informed Consent

Informed consent was obtained from all individual participants included in the study.

#### Conflicts of Interest Disclosure

The authors declare that they have no conflicts of interest.

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