REPRINTED FROM FEBRUARY 2025

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A 67-year-old female with rare intracranial mesenchymal tumor featuring the *EWSR1::CREM* fusion gene

CAP TODAY *and the Association for Molecular Pathology have teamed up to bring molecular case reports to* CAP TODAY *readers. AMP members write the reports using clinical cases from their own practices that show molecular testing's important role in diagnosis, prognosis, and treatment. The following report comes from New York Institute of Technology, Memorial Sloan Kettering Cancer Center, Good Samaritan Hospital, and St. Francis Hospital and Heart Center—all in New York. If you would like to submit a case report, please send an email to the AMP at* amp@amp.org. *For more information about the AMP and all previously published case reports, visit* www.amp.org.

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Intracranial mesenchymal tumors (IMT) are extremely rare and account for only 0.3 percent of soft tissue tumors.[1] Most frequently, mesenchymal tumors occur in subcutaneous tissue; they are rarely described in the central nervous system. In addition, these tumors typically occur in young adults,[1] with a median patient age of 14 (range: four to 70).[2]

Recently, it has become increasingly apparent that certain gene fusions may be shared among clinically unrelated sarcoma types. For example, the fusion genes *EWSR1::CREB1* and *EWSR1::ATF1* have been detected in angiomatoid fibrous histiocytoma, soft tissue and gastrointestinal clear cell sarcoma, primary pulmonary myxoid sarcoma, and hyalinizing clear cell carcinoma of the salivary gland.[3] Intriguingly, *EWSR1*-rearrangement mesenchymal tumors in the central nervous system are rare and were found in only a small number of adolescents and young adults with intracranial myxoid mesenchymal tumors.[4]

Approximately one-third of soft tissue tumors are characterized by specific recurrent chromosomal translocations, leading to the generation of aberrant chimeric transcription factors. Most fusion transcripts define the associated sarcoma and thus can be used as a highly specific molecular diagnostic marker under the right clinical and pathological context.[5]

IMT with a *FET::CREB* fusion gene can mimic other intracranial tumors radiologically and histologically. The extra-axial location as well as the presence of a dural tail can result in the impression of a meningioma or solitary fibrous tumor/hemangiopericytoma. They can also resemble a meningioma microscopically as well owing to the presence of syncytial growth, meningioma-like whorls, rhabdoid cytology, or the presence of cords of epithelioid cells in a mucin-rich stroma. The expression of desmin and the presence of a *FET::CREB* fusion gene are useful in distinguishing IMT from meningioma immunohistochemically[2] and molecularly.

Prior reports have demonstrated variable histopathological features including spindle cells in mucin or collagenous stroma, or epithelioid cells in a mucin-poor collagenous stroma. The immunohistochemical profile often demonstrates epithelial membrane antigen (EMA) and desmin positivity. One essential diagnostic feature defined by the World Health Organization classification is the intracranial location.[6]

The *EWSR1::CREM* fusion gene is a relatively novel variant of the *EWSR1::CREB* family of fusion genes, which was first identified in intracranial myxoid mesenchymal tumors (IMMT) in 2017 by Kao, et al.[3] This type of tumor present at the interhemispheric frontal region is a rare feature that has been reported in only three cases, all of which have the *EWSR1::CREM* fusion gene.[7] Here, we report a case of a 67-year-old female with the radiological presentation of a typical meningioma located in the anterior right frontal lobe. She was ultimately diagnosed with a myxoid spindle cell neoplasm that had characteristics of an IMT with the *FET::CREB* fusion gene.

Case presentation. A 67-year-old female presented to the emergency department in August 2023 after a witnessed seizure characterized by tonic-clonic movements and unresponsiveness for two to three minutes. She then experienced a brief respite followed by a second seizure.

The patient was postictal on arrival to the emergency department but hemodynamically stable.

The patient underwent radiological assessment first with head CT scans without contrast, followed by MRI scans with and without contrast. The axial noncontrast head CT (**Fig. 1A**) demonstrated a homogeneous mildly hypodense 1.9-cm mass, resulting in mild impression upon the right frontal lobe with associated mild vasogenic edema. An associated small dural tail, which is similar to a meningioma, was denoted on an axial post-contrast T1-weighted brain MRI (**Fig. 1B**). This was also noted on sagittal post-contrast T1-weighted brain MRI (**Fig. 1C**).

The patient subsequently underwent a right frontal craniotomy and brain tumor resection two days after the initial presentation. An intraoperative consultation grossly revealed a pale pink, irregularly shaped soft tissue mass measuring 1.3×0.3 cm. Microscopically, lesional tissue was reported as present. The excisional tissue was sent to Memorial Sloan Kettering Cancer Center for further workup. The tumor was described as a pale pink, rubbery nodule measuring 1.6×1.3×1.2 cm; one surface was slightly smooth, and the opposite surface was rough and irregular. The cut surface had a homogeneously pale pink appearance.

MSKCC analyzed the specimen microscopically and performed special staining and genetic testing via Archer analysis. Hematoxylin and eosin staining and light microscopy examination exhibited similar histopathology to tumors of this variant of IMT, which included solid and epithelioid features. More commonly, these tumors have been described as composed of spindle cell elements, in reticulated arrangements within a conspicuously myxoid matrix.[8] The H&E staining showed a low-grade neoplasm with many spindle cells in a reticulated pattern present within a prominent myxoid background (**Fig. 2A**). Histopathology was reported to be consistent with a myxoid spindle cell neoplasm. Immunohistochemistry of the tumor showed the positive expression of desmin (**Fig. 2B-i**), EMA (**Fig. 2B-ii**), and CD99 (**Fig. 2B-iii**). The remaining immunohistochemical profile was negative for PR, SSTR2, SMA, STAT6, and MUC4 (data not shown). Ki-67, a proliferation marker for cancer cell division, was five percent (data not shown).

MSKCC also performed the MSK-Fusion assay to determine whether there is a known fusion gene in the

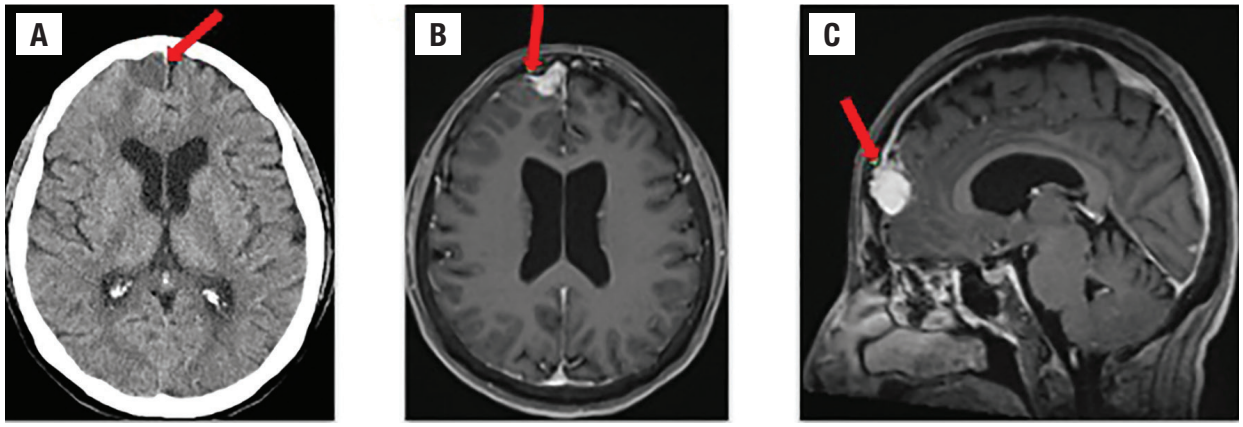

Fig. 1. Preoperative radiological images. **A)** Axial noncontrast head CT with the red arrow denoting the anterior right frontal convexity, extra-axial, homogeneous mildly hypodense 1.9-cm mass, resulting in mild impression upon the right frontal lobe. **B)** Axial post-contrast T1-weighted brain MRI demonstrating the anterior right frontal convexity, extra-axial, avidly enhancing mass, with the red arrow denoting an associated small dural tail (which is similar to meningioma). **C)** Sagittal post-contrast T1-weighted brain MRI with the red arrow pointing to the anterior right frontal convexity, extra-axial, avidly enhancing 1.9-cm mass, resulting in mild impression upon the right frontal lobe.

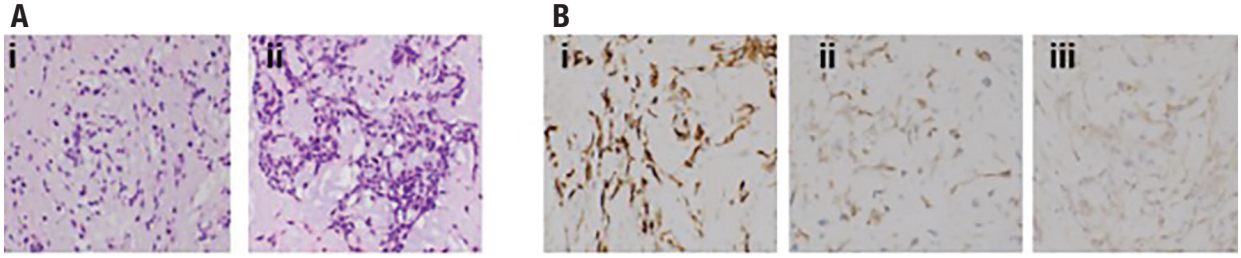

Fig. 2. Histopathological and molecular analysis of the patient's resected tumor. **A-i)** Hematoxylin and eosin (H&E) staining of the patient's tumor cells showed low-grade spindle-shaped cells with light pink cytoplasm and extracellular matrix myxoid, spindle cell tumor at 20×. **A-ii)** Focus of reticulated growth pattern at 20×. **B)** Immunohistochemistry analysis of the patient's resected tumor cells. The patient's tumor cells demonstrated strong desmin expression **(B-i)**, EMA expression **(B-ii)**, and CD99 expression **(B-iii)**.

patient's tumor, which may provide a more definitive diagnosis (**Fig. 3A**).[8] Briefly, RNA was extracted from formalin-fixed, paraffin-embedded tumor tissues and quantified. cDNA library was prepared from the extracted RNA using the Anchored Multiplex PCR technology and custom-designed gene-specific primers and sequenced. Fusion genes were identified using the Archer analysis software. Excitingly, this genetic workup demonstrated the presence of the *EWSR1::CREM* fusion gene (**Fig. 3B**).

Collectively, the final pathology report designated this as a myxoid spindle cell neoplasm characteristic of intracranial mesenchymal tumor with the fusion gene *FET::CREB*.

Postoperative imaging demonstrated a small resection cavity in the anterior right frontal region with reactive dural enhancement (data not shown). In December 2023, the patient had follow-up imaging for evaluation of the anterior frontal resection site. This revealed the presence of underlying encephalomalacia/gliosis. It was also noted that there was near resolution of fluid underlying the craniotomy with no new mass-like enhancement. Parenchymal volume loss and mild microvascular ischemic changes were also reported. The CT scan of the chest, abdomen, and pelvis showed no evidence of metastatic disease (data not shown). The patient's 10-month postoperative surveillance MRI did not show any evidence of recurrence (data not shown). The patient is on antiseizure medication (levetiracetam) and has been neurologically stable without any further seizure episodes. She continues to follow up with a neurologist. The patient is doing well per her provider and will continue image surveillance.

Discussion. A few cases of intracranial mesenchymal tumors have been reported and have been variously termed intracranial angiomatoid fibrous histiocytoma or intracranial myxoid mesenchymal tumor.[2] Prior case reports discussing intracranial mesenchymal tumors have disclosed both similarities with and differences from this case report. In 2017, Kao, et al., first described the novel *EWSR1::CREM* fusion gene, a variant of the *EWSR1::CREB* family of gene fusions.[3]

Most intracranial mesenchymal tumors have been reported in children and young adults in the second and third decades of life, with a median age of 17.[2] Intriguingly, our case report is of a 67-year-old woman. Overall, females were affected more frequently than males, with a male-to-female ratio of 1:1.8 in both pediatric and adult populations.[10] Prior tumors have been described as uniformly extra-axial or intraventricular with locations at the cerebral convexities, falx, lateral ventricles, tentorium, cerebellopontine angle, and spinal cord.[2] The location of our patient's neoplasm was designated as extra-axial and parafalcine, demonstrating similarities to prior cases.

Radiological reports have shown an extra-parenchymal lobulated mass without a dural tail,[6] choroid plexus (glomus choroideum) meningioma,[9] and contrast-enhancing, surrounding vasogenic edema, with no definitive dural attachment.[11] The radiologic report of our patient's tumor similarly showed vasogenic edema within the right frontal lobe; however, in contrast, a dural tail anteromedial to the right frontal lobe was reported, which gave the impression of a meningioma at first.

Histopathological analysis has varied among case reports and has revealed epithelioid morphology with mucin-rich stroma[6] mostly showing "myxoid and/or fibromyxoid background stroma, with a variety of tumor architectural patterns, including cords and sheets, and diverse cell

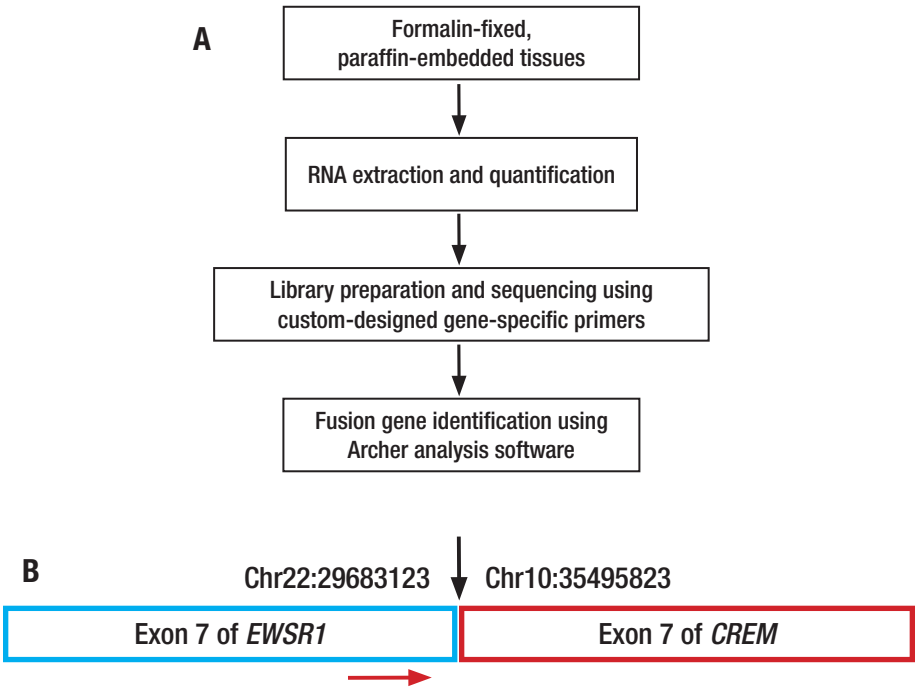

Fig. 3. Identification of the *EWSR1::CREM* fusion gene through MSK-Fusion. **A)** A workflow of the MSK-Fusion. **B)** The fusion gene analysis was done at Memorial Sloan Kettering Cancer Center. The *EWSR1::CREM* fusion event was called by the Archer FusionPlex analysis pipeline. There are 173 supporting reads with 53 different start sites for the fusion event. The in-frame status of the fusion is True, which indicates the fusion is functional. Both partner genes *EWSR1* and *CREM* have been known to be present according to Quiver database. The red arrow below the *EWSR1* gene represents the gene-specific primer and direction within the gene panel design. The vertical black arrow indicates the breakpoint of the fusion gene, which is at Chr22:29683123:Chr10:35495823.

morphology, including epithelioid, rhabdoid, and stellate/spindle cell." Additionally, histopathology reports have shown collagenous or myxoid stroma; internodular septae; lymphoplasmacytic cuffing; and hemangioma-like, or staghorn/hemangiopericytoma-like, vasculature.[2,9] Our patient's tumor demonstrated myxoid background stroma with spindle cells. Immunohistochemistry studies of prior cases have also shown positive expression of EMA and desmin,[6] EMA and CD99,[9] or desmin, EMA, CD99, MUC4, and synaptophysin.[2] Similarly, our patient's tumor showed the expression of EMA, CD99, and desmin (Fig. 2B).

In terms of genetic fusions, most intracranial mesenchymal tumors have shown the presence of a chimeric fusion of a *FET* family gene, usually *EWSR1*, to a *CREB* family transcription factor of *ATF1, CREB1*, or *CREM*.[2,6,12] A report of an *EWSR1::CREM* fusion demonstrated an epigenetic DNA-methylation profile.[6] Based on the genetic profiling done on the tumor tissue of our case, the tumor was positive for *EWSR1::CREM* fusion (Fig. 3).

Studies have shown that a chromosomal translocation producing the *EWSR1::CREM* fusion gene and a chimeric protein has resulted in divergent malignancies including both low-grade to highly malignant cancers. The molecular mechanisms related to the chimeric protein's oncogenic properties were studied using siRNA-mediated depletion of the chimeric EWSR1-CREM protein and showed altered expression of 712 genes. Many of these genes were found to be involved in cell cycle, proliferation, and migration.[13]

Ewing sarcoma breakpoint region 1 (*EWSR1*) gene is located on chromosome 22q12 and contains 17 exons, which encodes a highly conserved protein with 656 amino acid. EWSR1 protein belongs to the FET family of proteins, which also includes FUS and TAF15. This family of proteins is a highly conserved group of multifunctional, RNA-binding proteins that play a role in regulating transcription, RNA processing, mRNA cytoplasmic destination, DNA repair mechanisms, meiotic and mitotic cell division, and cellular aging. They have been shown to promote various sarcomas if chromosomally rearranged.[14,15]

Cyclic adenosine monophosphate (cAMP) responsive element modulator (*CREM*) is a gene located on chromosome 10p11.21.[2] *CREM* encodes a protein that is part of the CREB family of transcription factors, including ATF1 and CREB.[9] CREM binds to the cAMP responsive elements. Through alternative promoter and translation initiation sites, CREM can exert spatial and temporal specificity to cAMP responsiveness. *CREM* gene has been found to produce various protein isoforms through alternative splicing, which can act as transcription activators or repressors.[16]

The long-term outcome of IMT patients with the *FET::CREB* family of gene fusions has varied. Local recurrence was common, ranging from six months to 120 months,[7] with an average of 12 months, and a median overall survival greater than 60 months.[2] This patient had a follow-up imaging four months after resection; no recurrence was noted at that time.

In terms of treatment, of the 44 cases that have been reported, 34 individuals underwent a gross total resection. Additionally, seven individuals underwent certain types of adjuvant therapy including chemotherapy, radiation therapy, or both.[10]

A previous study by Kaprio, et al., using siRNA-mediated depletion of EWSR1-CREM demonstrated the importance of the loss of ornithine decarboxylase 1 (ODC1), which is critically involved in polyamine synthesis.[13] Polyamine biosynthesis is a rate-limiting step that regulates cell proliferation. The results from this study confirmed that the oncogenic properties of EWSR1-CREM are likely mediated by ODC1. These findings provide novel insights into the pathogenesis of tumors harboring an *EWSR1::CREM* fusion gene, hopefully facilitating the development of novel therapeutic strategies. For example, allicin, a reactive sulfur species from garlic (Allium sativum L.), is hydrophobic in nature and can efficiently cross cellular membranes. Schultz, et al., reported that allicin targeted ODC1 in neuroblastoma cells.[17] Remarkably, allicin inhibited the enzyme activity with a 23,000-fold higher potency than difluoromethylornithine.[18] This demonstrates a potential therapeutic use for allicin in treating IMT.

In summary, we report a novel intracranial mesenchymal tumor with the *EWSR1::CREM* fusion gene that has similarities to and differences from prior case reports of this tumor type. This case is even more unique based on the older age of our patient. IMT is a rare entity that may present with diagnostic challenges owing to its rarity and its close resemblance to other tumors. While this report has the potential benefit to aid in future diagnoses of similar cases, continued research is still needed. □

Abbreviations: EWSR1: Ewing Sarcoma Breakpoint Region 1 Gene; CREM: cAMP Responsive Element Modulator; CREB: cAMP Response Element Binding Protein; ATF1: Activating Transcription Factor 1; FET: FUS/EWS/Taf15 fusion oncoproteins; FLAIR: Fluid Attenuated Inversion Recovery; CD99: Cluster of Differentiation 99; PR: Progesterone Receptor; SSTR2: Somatostatin Receptor 2; SMA: Smooth Muscle Actin; STAT6: Signal Transducer and Activator of Transcription 6; MUC4: Mucin 4.

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Test yourself

Here are three questions taken from the case report. Answers are online now at www.amp.org/casereports *and will be published next month in* CAP TODAY.

1. Intracranial mesenchymal tumor can present as what on radiological imaging?
a. Glioblastoma multiforme
b. Meningioma
c. Pilocytic astrocytoma
d. Ependymoma

2. What is the typical age of patients who present with intracranial mesenchymal tumors?
a. Child to adolescence: 0–18 years
b. Young adult to adult: 19–59 years
c. Senior adult: 60 years and above
d. a and b
e. b and c
f. a and c

3. A patient who presents with seizures and imaging reveals an extra-axial mass in the anterior right frontal area, later diagnosed as a myxoid spindle cell neoplasm with an *EWSR1* fusion gene. Which of the following features is typical of this type of tumor?
a. A calcified mass with significant necrosis
b. A homogeneous mass with a small dural tail and low mitotic activity
c. A highly cellular mass with marked pleomorphism
d. A cystic mass with extensive inflammation