Letter

A novel FUS::BEND2 fusion expanding the molecular spectrum of astroblastomas

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Introduction

The name astroblastoma was modified to include "*MN1*-altered" in the latest World Health Organization (WHO) Classification of Tumors of the Central Nervous System (2021) [1], after the discovery of *MN1* fusions (with *BEND2* as the main partner gene) [2]. The presence of this alteration constitutes an essential diagnostic criterion for this tumor type, and for unresolved lesions, a DNA-methylation profile is necessary to confirm this diagnosis. The recent literature has concluded that *BEND2* fusions (without the *MN1* gene) may occur in this tumor type. Herein, we report one case of astroblastoma with a novel *FUS::BEND2* fusion. We compare its clinical, histopathological, immunophenotypic, genetic, and epigenetic features with those previously described in astroblastomas, *MN1* and/or *BEND2* fusion-positive.

Case presentation

In 2007, a 4-year-old girl presented with a hyperdense and calcified mass extending from the third to the fourth ventricle with hydrocephalus and intraventricular hemorrhage (**Figure 1 A–B**). The tumor was partially resected, and a diagnosis of ependymoma was made according to the 2007 WHO Classification. The tumor recurred 14 months later, and the patient died 69 months after the initial diagnosis. In 2024, a review of the diagnosis was performed. Microscopically, the re-examination

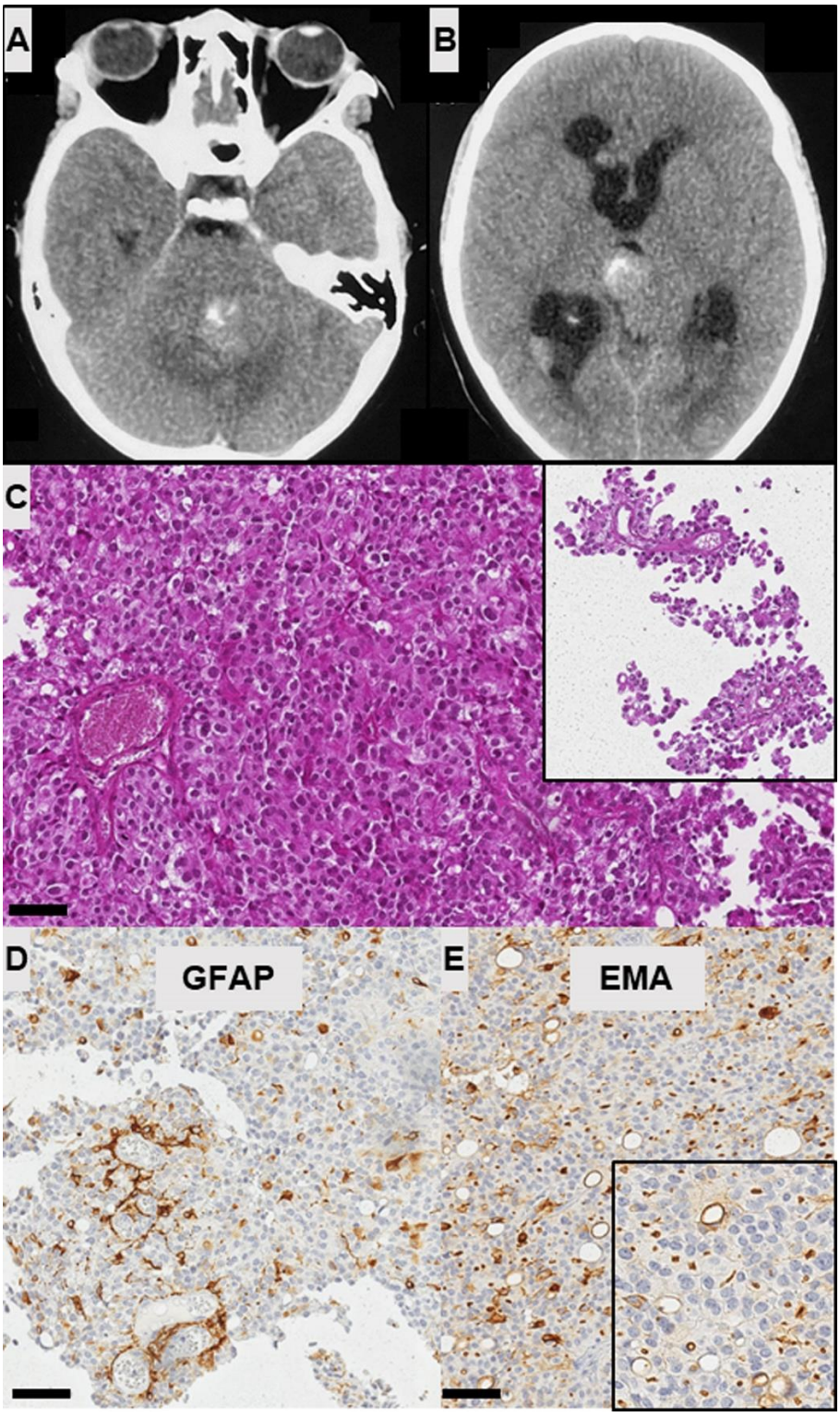

Figure 1. Radiological and histopathological features of the tumor (**A–B**) Axial unenhanced Computerized Tomodensitometry images of the patient, showing a hyperdense and calcified mass extending from the third to the fourth ventricle with hydrocephalus and intra-ventricular hemorrhage. (**C**) An ependymoma-like tumor with astroblastic pseudorosettes (HPS, magnification x100 and x400 for insert). (**D**) Expression of GFAP by tumor cells (magnification x400). (**E**) EMA immunoexpression by tumor cells (magnification x400).

Scale bars represent 60 µm for figures C–E.

HPS: Hematoxylin Phloxin Saffron.

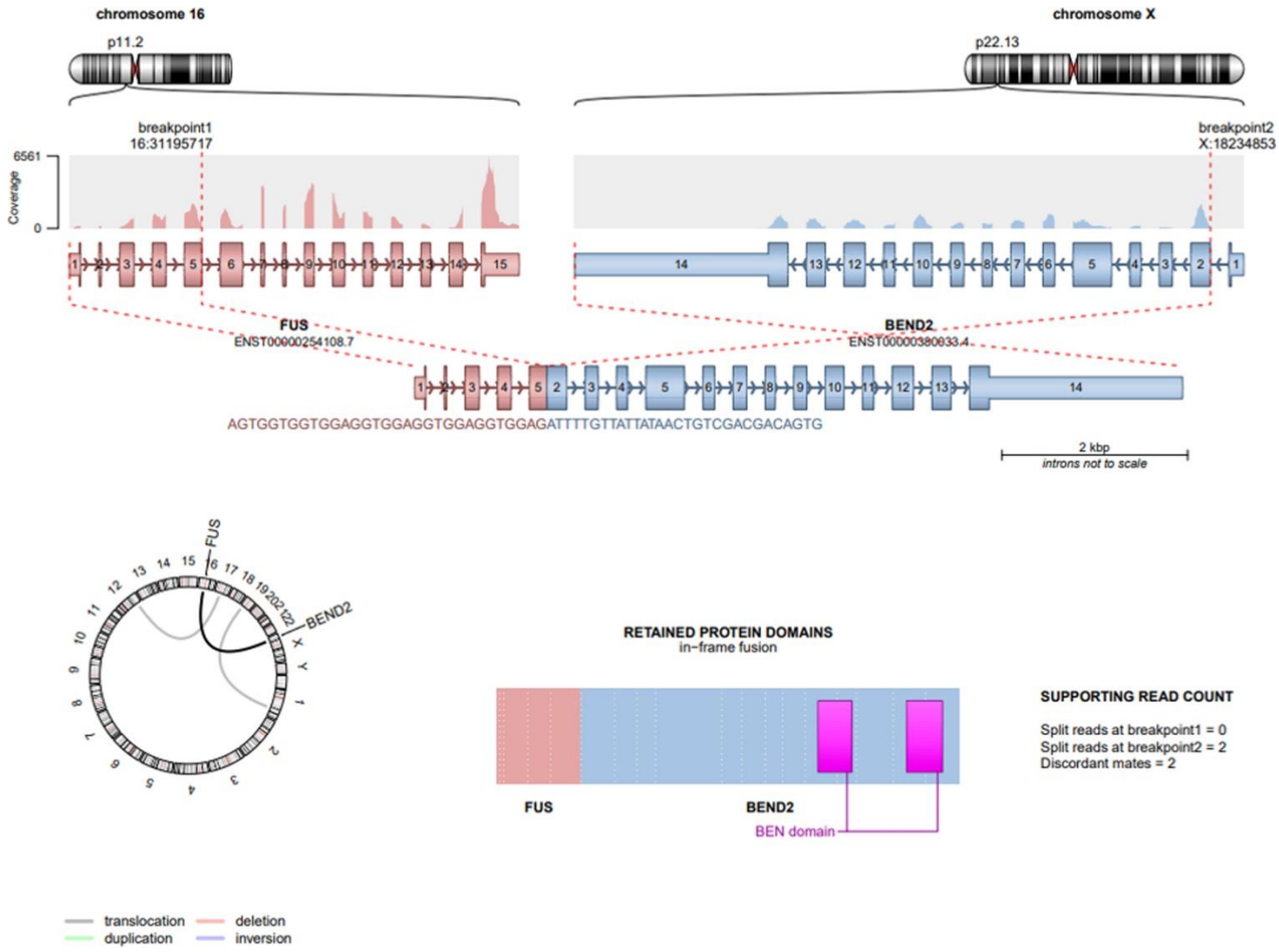

Figure 2. Genetic features (**A**) RNAseq analysis highlights a fusion between *FUS* (pink) and *BEND2* (blue) genes, respectively located on chr16p11.2 and chrXp22.13. (**B**) Chimeric protein between FUS and BEND2 with two retained BEN protein domains of BEND2.

showed a tumor having an ependymoma-like pattern with astroblastic pseudorosettes (**Figure 1C**). There was no clear cell component, no hyalinized fibrous stroma nor PAS-positive eosinophilic granular bodies. There was no microvascular proliferation or necrosis. Mitotic figures were scarce, and the MIB1-labeling index was low (5 %). The expression of BRG1, INI1 and H3K27me3 was retained. The tumor cells expressed GFAP and EMA with a dot-like and a microlumen pattern (**Figure 1 D–E**). There was no immunoreactivity for OLIG2, CK18, LIN28A, NFκB, L1CAM or neurofilaments. FISH analysis of the *MN1* gene failed to reveal a rearrangement, and RNA sequencing evidenced a *FUS::BEND2* gene fusion (**Figure 2A–B**). Additionally, DNA-methylation analysis was conducted. The tumor was not classifiable using the Heidelberg Brain Tumor Classifier (v12.8) and the Bethesda Brain Tumor Classifier (v2), but clustered within astroblastoma, *EWSR1::BEND2* methylation class using the uniform manifold approximation and projection (UMAP) method (**Supplementary Figure 1**). Because of the histopathological features and the presence of a *BEND2* fusion, the integrated diagnosis was an astroblastoma, *BEND2* fusion-positive.

Discussion and conclusions

The current literature review includes 92 cases of astroblastomas that either carried a *MN1* fusion or had a methylation profile compatible with astroblastoma, *MN1*-altered [2–28] (see **Supplementary Table 1** for literature review). The majority of cases are supratentorial (84 %, 72/86 tumors with available data) [2–28]. Most patients are pediatric (78 %,

67/86 cases with available data) [2–28]. However, this tumor occurs in a wide age range (0–60 years) [11,23]. Typical histopathological features include an ependymoma-like pattern, astroblastic pseudorosettes and stromal sclerosis. As ependymomas, tumor cells frequently express GFAP [3,4,6–11,14–19,22–25,27] and EMA [3,4,6,8–11,14,16–19,21,22,25,28] with a variable OLIG2 immunopositivity [3,4,7,9–11,11,13,15–17,19,21–25,28]. A *MN1* gene fusion is found in 84 % of astroblastomas (54/64 reported cases with genetic analyses, the 28 remaining cases being only diagnosed using DNA-methylation profiling analysis), mainly in association with the *BEND2* gene (94 %, 17/18 cases with RNA-sequencing data, the 36 remaining cases being only diagnosed using FISH *MN1*) [2–4,6–16,18–28]. One tumor harbored a *MN1::CXXC5* fusion [2]. Alternative fusions including the *BEND2* gene without *MN1* have been reported in ten tumors (7 with *EWSR1,* 2 with *MAMLD1,* and one with *YAP1*) [3,4,8,19,22,23,27]. Moreover, an *EWSR1::EZHIP* fusion was identified in an astroblastoma; however, no methylation-based classification was available for this tumor [29]. In this study, we report for the first time a *FUS::BEND2* fusion in astroblastoma. This novel gene rearrangement results in an in-frame fusion of exons 1 to 5 of the *FUS* gene and exons 2 to 14 of the *BEND2* gene, the same breakpoint as previously reported [3,4]. In the literature, we identified two reports of a nasopharyngeal and soft tissue sarcoma harboring a *FUS::BEND8* (another gene encoding a BEN domain containing protein) fusion [30,31]. The *FUS* gene belongs to the FET family of genes which encode for RNA-binding proteins, like *EWSR1* [32]. The predicted FUS-BEND2 chimeric protein maintained two BEN domains in the C-terminus encoded by *BEND2,* which are involved in transcription and chromatin regulation [33].

As was found for our case, clinical findings seem to be different for patients who have an astroblastoma, *BEND2* fusion-positive. They tend to present in a younger population (median age of 6 years-old at diagnosis), affect females less frequently (with a female-to-male ratio of 1.75), and be mainly infratentorial (73 % of cases) compared to the astroblastomas, *MN1*-altered (presenting at a median age of 10 years, a female-to-male ratio of 7.5 and only making up 8 % of infratentorial tumors) [2–4,6–16,18–28]. Most likely due to the tumor location, the prognosis of patients with astroblastomas, *BEND2* fusion-positive is worse (33 %, 3/9, patients died with a median overall survival of 25 months [1;276]) than those with astroblastomas, *MN1*-altered (14 % of patients, 9/62, died with a median overall survival of 70 months [3;324]). The analysis of the recent literature seems to suggest that it may be *BEND2,* rather than *MN1,* which has a more critical functional role in the oncogenesis of astroblastomas [34].

In conclusion, we expanded the astroblastoma genetic spectrum with one novel fusion *FUS::BEND2*. Reminiscent of the terminological history of supratentorial ependymomas, originally referred to as "*RELA*-fusion positive" and afterward "*ZFTA*-fused", our case supports the consideration of a *BEND2* fusion as the defining alteration for astroblastoma, rather than the *MN1* rearrangement.

Ethics approval

This study was approved by the GHU Paris Psychiatrie Neurosciences, Sainte-Anne Hospital's local ethic committee.

Consent for publication

The patient signed informed consent forms before treatment was started.

Competing interests

The authors declare that they have no conflicts of interest directly related to the topic of this article.

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Authors' contributions

ATE, VDR, KB and NB compiled the MRI and clinical records; ATE, AM and PV conducted the neuropathological examinations; BB conducted the molecular studies; ATE, LH, and PV drafted the manuscript. All authors reviewed the manuscript.

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Supplementary material

Supplementary Table 01 (download) - Literature review (PDF)

Supplementary Figure 01 (download) - Uniform manifold approximation and projection (UMAP) analysis (PDF)

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