

## BRIEF COMMUNICATION

# Long survival in a child with a mutated K27M-H3.3 pilocytic astrocytoma

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## Introduction

Pilocytic astrocytoma is the most common brain tumor in children. It is a well circumscribed tumor with a slow growth and is classified as a grade I tumor by the World Health Organization.<sup>1</sup> Malignant transformation (MT) of low-grade gliomas (LGG) is a very unusual event in the pediatric population.<sup>2</sup>

H3.3 K27M and G34V/R histone mutations were recently identified as molecular drivers of a subgroup of pediatric high-grade gliomas (HGG), and the H3.3 K27M mutation is associated with worse overall survival.<sup>3,4</sup>

Here, we describe the first case of spontaneous MT of a H3.3 K27M mutated cervical spinal cord WHO grade I juvenile pilocytic astrocytoma (JPA) to glioblastoma, after a symptom-free period of 10 years in a teenager. Interest-

## Abstract

We report the first case of a child with a H3F3A K27M mutated pilocytic astrocytoma, who presented with a 10 years survival, and underwent spontaneous malignant transformation. The complex tumoral chromosomal rearrangements were consistent for genomic instability and for the histopathological features of malignant transformation into glioblastoma. H3F3A K27M mutations are rarely observed in benign neoplasms and may be associated with an adverse outcome. This mutation might not be the major driver that led to the onset of tumorigenesis, and we could consider that the associated *TP53* mutation, would be required for malignant transformation.

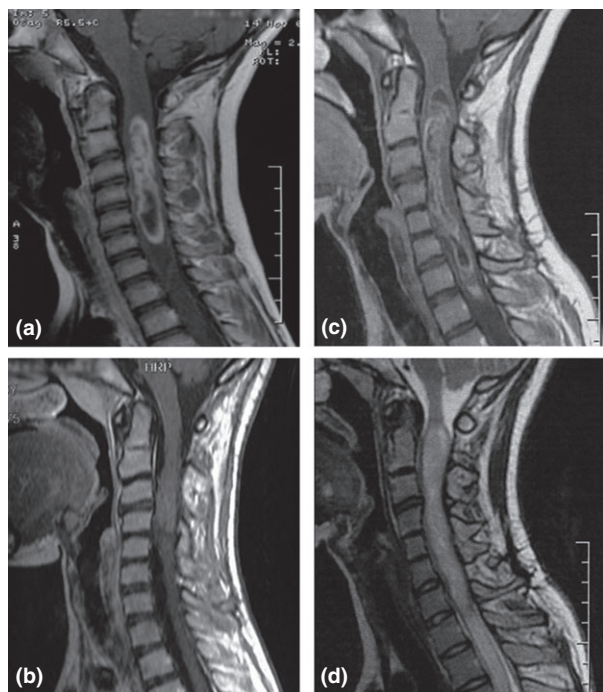
ingly, the H3.3-K27M mutation was already present at diagnosis and could explain the unusual evolution of the disease.

## Patient and Methods

### Case report

A 7-year-old girl with no familial history of neurofibromatosis presented with torticollis and left hemiparesia in 2002. Sagittal T1 MRI revealed a well-defined heterogeneous tumor of the cervical spinal cord from level C2, down to C6, with solid and cystic tumor parts and marked contrast enhancement (Fig. 1).

Complete surgical removal of the tumor was performed. Clinical, radiological, histological and immun-



**Figure 1.** MRI from the first tumor in 2002 (left column) and the recurrence in 2012 (right column). (A) Initial MR Imaging at diagnosis in November 2002, sagittal postcontrast T1 spin echo weight imaging (TR: 400; TE: 16) showing a heterogeneous tumor of the cervical spinal cord from level C2, down to C6, with solid and cystic tumor parts and with marked contrast enhancement. (B) Three years postsurgery treatment, sagittal MRI postcontrast T1 weight imaging (TR: 463; TE: 10): no recurrence. (C) Ten years after surgery treatment, postcontrast sagittal T1 weight imaging (TR: 470; TE: 8.6) showing a relapse as a spinal expansion from the cranio-cervical junction to level T1, with heterogeneous enhancement. (D) Ten years after surgery treatment, sagittal T2 weight imaging (TR: 3405; TE: 122) showing relapse with a hyperintense signal down to level T4.

ohistochemical data were consistent for WHO grade 1 pilocytic astrocytoma.

The patient received no further treatment. Regular MRI after tumor removal showed no residual tumor.

Ten years later, in 2012, the patient presented with rapidly evolving neck pain, worsening of her hemiparesis and balance disorders.

Spinal MRI revealed a tumor relapse expanding from the cranio-cervical junction to level T1, with a hyperintense signal on T2 weight imaging and heterogeneous enhancement on postcontrast T1 weight imaging. There was also a hyperintense signal on T2 weight imaging down to level T4. Brainstem was normal and no metastatic dissemination was observed. Surgical resection was subtotal, and the tumor was diagnosed as a WHO grade IV glioblastoma.

The patient underwent radiation therapy on the whole neuroaxis (40 Gy in 20 sessions with focal boost of  $2 \times 5$  Gy on tumor site) plus concomitant daily temozol-

omide, followed by maintenance temozolomide. Five months later, the patient received irinotecan and bevacizumab due to tumor progression. Finally, the patient died 12 months later.

### Histological and immunohistochemical examinations

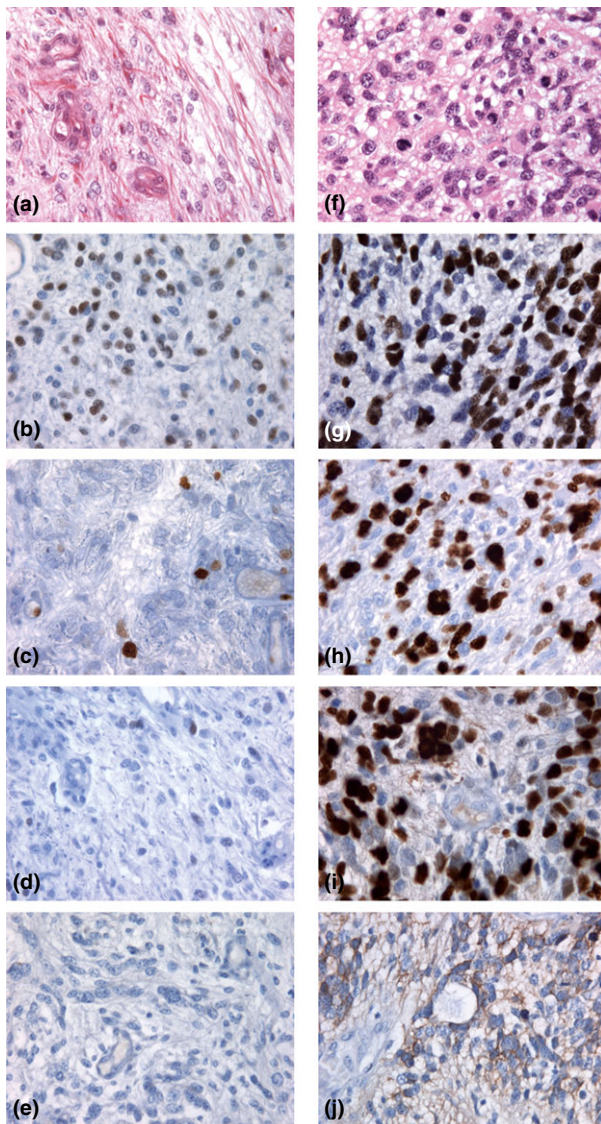
In 2002, light microscopy examination revealed that the first tumor tissue fit with the criteria of a biphasic, WHO grade 1 pilocytic astrocytoma with only slight nuclear atypia. Rosenthal fibers and granular eosinophilic bodies were prominent in the most abundant piloid component, whereas oligodendroglioma-like fields were not so obvious. Mitoses were rare; there was a marked glomeruloid vascular proliferation. Necrosis was barely observed. Olig2 staining was positive, and CD34 immunohistochemistry was negative. The tumor was circumscribed, and synaptophysin labeling failed to show any neurites within the tumor tissue. Furthermore, 5% of the nuclei were immunoreactive for Ki67, and only rare nuclei exhibited a faint labeling of p53; EGFR (Epidermal Growth Factor Receptor) was negative.

Microscopic features of a glioblastoma were present at examination of the tumor that was resected at recurrence in 2012 (Fig. 2). Tumor cells had a stellate or elongated morphology, with a brisk mitotic activity, necrosis and vascular festoons. They were labeled by anti-Olig2 (70%) but not with anti-IDH1 (R132H) antibodies, and the Ki67 index reached 30% of the tumor cell nuclei. In addition, p53 immunoreactivity was strong, and 20% of the cell tumors were EGFR-immunoreactive. CD34 labeling remained negative. Immunofluorescence staining revealed a reduction in H3K27 methylation.

### Molecular data

After genomic DNA extraction, a mutation analysis of hotspot codons 27 and 34 of the *H3F3A* gene was investigated using pyrosequencing (Qiagen, Valencia, CA, USA). Exon 4 of the *IDH1/2* genes, exon 15 of the *BRAF* gene and all of the exons of the activin A receptor type I (*ACVR1*) and *TP53* genes were also analyzed using Sanger sequencing (Applied Biosystems, Foster City, CA, USA). Unexpectedly, these results showed the exclusive presence of a K27M (c.83A>T, p.Lys27Met) H3.3 mutation in the primary tumor from 2002. In the tumor recurrence from 2012, K27M mutation associated with a *TP53* mutation (c.817C>T, p.Arg273Cys) were detected (Fig. 3). No *IDH1/2*, *PTEN*, *BRAF*, and *ACVR1* mutations were found in both tumors.

Array comparative genomic hybridization (aCGH) was performed using a surePrint G3 Human CGH Microarray kit 60 K (Agilent Technologies, Santa Clara, CA, USA).



**Figure 2.** Light microscopy data from the first tumor sample resected in 2002 (left column) and the recurrence sample (right column), showing routine hematoxylin–erythrosin–saffron staining (first line) and immunohistochemistry of Ki67, EGFR, Olig2 and p53 (lines 2–5). (A and F) the first tumor exhibited hallmarks of an pilocytic astrocytoma, that is, piloid cells, Rosenthal fibers, eosinophilic granular bodies, and glomeruloid vascular proliferation, whereas in the second tumor, the cells were poorly differentiated. There was severe nuclear atypia and numerous mitoses. (B and G) Olig2 immunoreactivity is present in both tumors but is stronger in the recurrence. (C and H) The Ki67 index was 5% in the first tumor, but reached an overall 30% on the recurrence with a foci of 70%. (D and I) p53 labeling was faint in the first tumor, but strong in the second tumor (70% of the stained nuclei). (E and J) EGFR labeling appeared in the second tumor, as a membrane signal on 20% of tumor cells, but is weak to intermediate in intensity.

Profiles of aCGH showed no chromosomal abnormalities in the tumor from 2002, whereas the specimen from 2012 demonstrated multiple chromosome rearrangements with

specific losses in large chromosomal regions in 5q, 8, 9q, 10, 12q, 13q, 14, 16q, 17, 18q, and 21 and a gain of 9p (Fig. 3). There was no loss of 16p. Focal high copy number amplification was observed in segments, including the PDGFR (Platelet-Derived Growth Factor Receptor), EGFR, and MDM2/CDK4 genes.

To search for BRAF-KIAA1549 fusion transcript status (KIAA1549-Ex16\_BRAF-Ex9, KIAA1549-Ex15\_BRAF-Ex9, KIAA1549-Ex18\_BRAF-Ex11, and KIAA1549-Ex16\_BRAF-Ex11), total RNA was extracted and reverse transcriptase amplification chain reaction was performed. No KIAA-BRAF transcripts were detected.

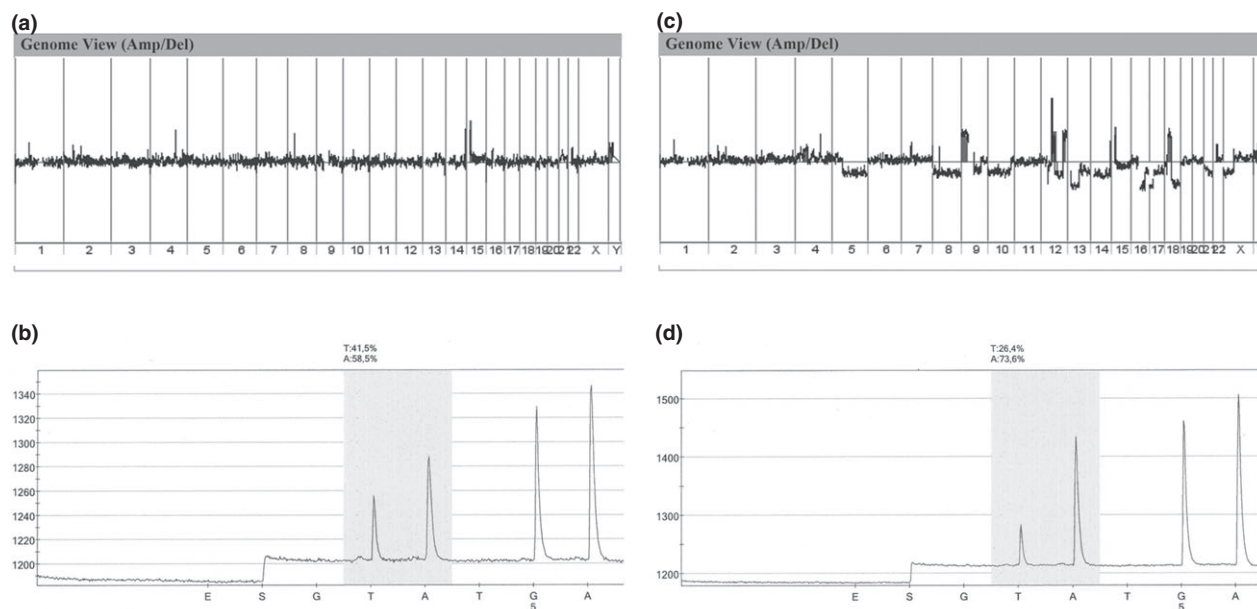
## Discussion

To the best of our knowledge, we report here the first case of a long survival of a child with *H3F3A* K27M mutated JPA undergoing spontaneous MT into glioblastoma.<sup>5</sup>

According to the WHO classification,<sup>1</sup> this patient initially had a pilocytic astrocytoma. Despite an unusually high Ki67 proliferation index, the long-term clinical course confirmed a benign slow-growing tumor. Expectedly, in our case, molecular analysis of the JPA did not disclose any alteration associated with circumscribed gliomas, that is, *BRAF-KIAA 1549* fusion, V600E BRAF mutation and IDH1/IDH2 mutation. Indeed, *BRAF-KIAA 1549* fusion mainly focuses on specific locations as infra-tentorial and optical pathway pilocytic astrocytomas.<sup>6</sup> The V600E BRAF activating mutation is commonly observed in supratentorial pediatric LGGs, mainly pleomorphic xanthoastrocytomas, gangliogliomas and extra-cerebellar pilocytic astrocytoma.<sup>6,7</sup> IDH1/IDH2 mutations are unusual in pediatric LGGs.<sup>2</sup>

MT of LGG is rarer in children than in adults<sup>2</sup> (6.7% vs. 50%, respectively). The main risk factors suspected of malignant degeneration are ionizing radiation and tumorigenesis predisposition syndrome. In a previous pediatric study of 11 patients, this event was mainly related to an infiltrative astrocytoma grade II WHO that degenerated into a glioblastoma, with a median latency of 5.1 years, and was most likely related to treatment for most of the patients.<sup>8</sup> Only three cases of spontaneous malignant degeneration from JPA to full blown glioblastoma have been previously reported in children.<sup>8–10</sup> All of the tumors were located intracranially, two received prior radiotherapy and one patient had neurofibromatosis type I. This patient presented with a thalamic tumor degenerating in less than 2 years into anaplastic astrocytoma, which led to a discussion of the diagnosis.<sup>8–10</sup>

The K27M H3F3A mutation was present not only in the high-grade recurrence tumor but also in the initial pilocytic astrocytoma, 10 years prior to the recurrence.



**Figure 3.** aCGH and *H3F3A* K27 mutational status in the first tumor sample resected in 2002 (left column) and the recurrence sample (right column). (A and C) Array comparative genomic hybridization (aCGH) genome plots indicating no aberration in the tumor of 2002 compared to the complex profile in 2012, which demonstrated multiple chromosome rearrangements with losses in large chromosomal regions in 5q, 8, 9q, 10, 12q, 13q, 14, 16q, 17, 18q, and 21 and gain of 9p. Focal high copy number amplification was observed in segments, including *PDGFR*, *EGFR* and *MDM2/CDK4* genes. (B and D) Representative pyrograms showing the K27M (wt AAG→ATG) mutation in tumor recurrence and in the primary tumor leading to an amino acid exchange from lysine (Lys) 27 to methionine (Met).

Histone H3.3 is a nuclear, replication-independent protein supporting DNA that is predominantly incorporated into transcription sites and associated with active and open chromatin. The K27M mutation is a gain-of-function mutation that impairs trimethylation at residue 27. H3.3 insertion is affected, and gene expression in the tumor cells is profoundly modified.<sup>3,4,11</sup>

The *H3F3A* K27M mutation is characteristic of midline HGG in children. In a previous study, it was shown that K27M-H3.3 is associated with worse prognosis in children with diffuse intrinsic pontine glioma, and all of the long-term survivors were wild type for *H3F3A*.<sup>4</sup> Here, we described long survival despite the K27M-H3.3 mutation. Zhang et al. already reported two patients with LGG (diffuse glioma) and K27M H3.3<sup>12</sup> and Jones et al. describes a patient with JPA and K27M H3.3 in association to *NF1* and *FGFR1*.<sup>13</sup>

In four previous studies, 34,<sup>3</sup> 12,<sup>14</sup> 29,<sup>12</sup> and 15,<sup>15</sup> JPA were investigated for the *H3F3A* K27M mutation: no mutation was found. Thus, these data questioned the prognostic role of this mutation in pediatric Central Nervous System (CNS) glial tumors. The K27M H3.3 mutation may not be exclusively related to high-grade tumors, and studies of the histone mutation should be performed routinely in all pediatric glial tumors. This could result in the identification of genetic subgroups of distinct histo-

logically indistinguishable entities, resulting in a better understanding the behavior of atypical tumors, as pilocytic astrocytoma with malignant features.

No somatic mutations in the gene *ACVR1* was found. These gain-of-function mutations occur in diffuse intrinsic pontine glioma in conjunction with the histone H3.1 p.Lys27Met substitution.<sup>16</sup> CGHa analysis performed in 2012 showed complex tumoral chromosomal rearrangements in comparison with 2002, which is consistent with the histopathological features of MT and genomic instability of glioblastoma.<sup>17</sup>

In our case of MT, several hypotheses may be made. First, the occurrence of relapse suggests that the initial tumor resection was microscopically incomplete, leaving a residual tumor containing cells carrying the K27M H3.3 mutation. The existence of this significant delay between the initial surgical resection and the appearance of glioblastoma suggests that, in this case, the K27M H3.3 mutation was the first event, although it was potentially not sufficient to lead to transformation. Thus, this mutation might not be the major driver that led to the onset of glioblastoma, and it is possible that another event, such as the second associated mutation in *TP53*, was responsible for the MT as *TP53* mutations are a crucial key in the cell cycle dysfunction. Another hypothesis could be that epigenetic events have suppressed the effect of the K27M H3.3 mutation.

Concerning the management of a similar tumor in 2014, we probably performed a more aggressive therapeutic approach with adjuvant chemotherapy or radiotherapy. This approach should have been suboptimal but unfortunately pharmacologic inhibition of histone demethylation is not currently available.<sup>18</sup>

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## Author Contributions

A. Hochart, F. Escande, N. Rocourt, C.-A. Maurage, and P. Leblond wrote the body of the manuscript. C.-A. Maurage and J. Beaujot provided tissue samples and performed histopathological analyses and performed validation experiments. S. Meignan produced figures. J. Grill, F. Escande, and V. Koubi-Pick performed mutation analysis and produced molecular data. All authors contributed to the final manuscript.

## Conflict of Interest

The ICMJE requirements for the authors have been met and the authors have nothing to report.

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