Activating NTRK2 and ALK receptor tyrosine kinase fusions extend the molecular spectrum of pleomorphic xanthoastrocytomas of early childhood: a diagnostic overlap with infant-type hemispheric glioma

Calixto-Hope G. Lucas1 · Zied Abdullaev2 · Carol S. Bruggers3 · Kanish Mirchia1 · Nicholas S. Whipple3 · Mouied M. Alashari4 · Amy Lowichik4 · Samuel Cheshier5 · Joanna J. Phillips1,6 · Patrick Devine7 · David A. Solomon1 · Martha Quezado2 · Kenneth D. Aldape2 · Arie Perry1,6

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Pleomorphic xanthoastrocytoma (PXA) is a circumscribed glioma arising in the cerebral hemispheres of children and young adults. Recent molecular studies demonstrate an activating mitogen-activated protein (MAP) kinase mutation (most frequently \(\text{BRAF} \text{p.V600E}\) hotspot mutation) with co-occurring homozygous deletion of \(\text{CDKN2A}\) encoding the \(\text{p16}\) cell cycle regulator protein in most PXA [11]. Less commonly, fusions involving \(\text{BRAF}\) and \(\text{RAF1}\) have previously been reported by our group [10]. Here, we present two young children with high-grade gliomas containing \(\text{CDKN2A/B}\) homozygous deletion and \(\text{NACC2–NTRK2}\) or \(\text{PPP1CB–ALK}\) fusion, along with DNA methylation signatures aligning to PXA. In both, the main differential diagnostic consideration was infant-type hemispheric glioma (IHG).

Patient #1: This 3-year-old boy presented with a 3-month history of progressive headaches, emesis, sound sensitivity and altered gait. Magnetic resonance imaging (MRI) demonstrated a 9.4 cm avidly enhancing, solid and cystic right temporal horn mass, extending into adjacent parenchyma and middle/posterior cranial fossa. He underwent gross total resection. Intraoperatively, the tumor appeared tan-yellow and vascular with extensive tentorial involvement. Histology revealed a mostly solid appearing spindled and epithelioid glial neoplasm arranged in fascicles, sheets, and papillae (Fig. 1a). Perivascular hyalinization was focally prominent and there were areas of palisading necrosis (Fig. 1b), microvascular proliferation, and up to 6 mitotic figures per 10 high-power fields. No definite eosinophilic granular bodies or Rosenthal fibers were seen. There were occasional multinucleate tumor cells. The tumor cells were extensively immunoreactive for \(\text{GFAP}\) (Fig. 1c), \(\text{OLIG2}\), and \(\text{CD34}\) (Fig. 1d), but negative for \(\text{BRAF V600E}\) mutant protein. There was no increased intercellular reticulin deposition. A neurofilament stain highlighted entrapped axons only at the tumor periphery and the p53 labeling index was 60%. A diagnosis of high-grade glioma was rendered with consideration of PXA, IHG, and astroblastoma. Additional molecular studies revealed \(\text{NACC2–NTRK2}\) fusion (Fig. 1e–f) and \(\text{CDKN2A/B}\) homozygous deletion (Supplementary Fig. 1 [Online Resource 1]). Post-operatively, the patient was treated with focal proton radiation to 55.8 Gy and concurrent temozolomide followed by 24 months of adjuvant daily oral larotrectinib (NTRK inhibitor) therapy. The patient is alive without evidence of disease 33 months following diagnosis.

Patient #2: This almost 3-year-old girl with longstanding mild gross motor and speech delays presented with a six-month history of episodic uncontrollable right arm shaking. MRI demonstrated a rounded 2.5 cm, partially cystic, enhancing left frontoparietal mass between the motor and
Patient #1, 3-year-old male with PXA harboring NACC2-NTRK2 fusion

Patient #2, 2-year-old female with PXA harboring PPP1CB-ALK fusion
sensory cortices. A gross total resection was achieved after two surgeries. The tumor was tan-and well demarcated from adjacent brain parenchyma. Sections revealed a predominantly solid glial neoplasm with cells containing enlarged, irregular hyperchromatic nuclei, including scattered multinucleated forms (Fig. 1g). Areas of necrosis, microvascular proliferation, and up to 6 mitotic figures per 10 high-power fields were noted. No definite eosinophilic granular bodies or Rosenthal fibers were seen. Immunohistochemical stains demonstrated patchy positivity for GFAP (Fig. 1h), with extensive OLG2, CD34, and ALK-positivity (Fig. 1i). The tumor cells were BRAF V600E negative and showed loss of p16 immunoreactivity (Fig. 1j). An initial diagnosis of high-grade glioma was rendered, with IHG (with a likely ALK alteration) and PXA being favored. Additional molecular studies revealed PPP1CB–ALK fusion (Fig. 1k–l) and CDKN2A/B homozygous deletion (Supplementary Fig. 1 [Online Resource 1]). Post-operatively, the patient (now age 3) was treated with focal proton radiation to 55.8 Gy and concurrent temozolomide followed by adjuvant lorlatinib (ALK inhibitor) therapy. The patient is alive without evidence of disease 6 months following diagnosis.

UCSF500 targeted next-generation sequencing (NGS) profiling was performed as previously described [7]. Tumor #1 demonstrated a chromosome 2p inversion event resulting in a fusion involving the 5’ end of NACC2 (exons 1–5 [transcript ID NM_144653]; Fig. 1e) and the 3’ end of NTRK2 (exons 15–21 [transcript ID NM_006180], encoding the tyrosine kinase domain; Fig. 1f). Tumor #2 demonstrated a chromosome 9q inversion event resulting in a fusion involving the 5’ end of PPP1CB (exons 1–7 [transcript ID NM_206876]; Fig. 1k) and the 3’ end of ALK (exons 20–29 [transcript ID NM_004304], encoding the intracellular kinase domain; Fig. 1l). Both also demonstrated homozygous CDKN2A/B deletion (Supplementary Fig. 1 [Online Resource 1]). No other structural variants, focal chromosomal amplifications or deletions were noted. For both, DNA methylation profiling was performed as previously described [12] and demonstrated close proximity to a PXA reference cohort using tSNE dimensionality reduction analysis (Supplementary Fig. 2 [Online Resource 1]), with calibrated scores of > 0.99 matching to the PXA methylation class using both the 11b4 and 12.3 versions of the online DKFZ random forest classification algorithm (molecular neuropathology.org). The integrated diagnosis for both was PXA, CNS WHO grade 3.

Given the young patient ages, the diagnosis of IHG was considered initially. This new entity in the 5th edition of the CNS WHO [2] presents in early childhood (< 1 year of age) with enrichment for receptor tyrosine kinase (RTK) fusions involving NTRK1/2/3, ROS1, ALK or MET [4, 5, 8]. The same PPP1CB–ALK fusion identified here has been described in two IHGs [1, 5, 9]. However, both patients were less than 1 year of age, and neither harbored CDKN2A/B homozygous deletion. NTRK family fusions have also been reported in rare pilocytic astrocytomas [6]. Of note, a pediatric PXA was reported to harbor an ETV6–NTRK3 fusion along with CDKN2A homozygous deletion [13]. The same NACC2–NTRK2 fusion with CDKN2A homozygous deletion identified here has recently been described in a pediatric cerebellar high-grade glioma [3]. Ancillary DNA methylation studies were not performed in these cases.

Given the non-canonical molecular profiles in our two cases, the integrated diagnosis was heavily weighted on the DNA methylation profiling results. These cases extend the molecular spectrum of anaplastic PXA to include tumors harboring CDKN2A/B homozygous deletion. NTRK family fusions have also been inconsistently detected using conventional NGS methods. Additional molecular studies could be informative to more accurately categorize these challenging pediatric lesions which may otherwise be classified as IHG or diffuse pediatric-type high-grade glioma, H3-wildtype, and IDH-wildtype according to contemporary criteria [2]. Although both of these patients demonstrate no recurrent disease following gross total resection and treatment with focal proton radiation followed by adjuvant NTRK and ALK inhibitor targeted therapy, this report is limited by short follow-up data. Patients with recurrent disease may benefit from personalized molecularly based therapeutic strategies and enrollment in precision medicine clinical trials. These cases underscore the importance of an integrated histologic and molecular approach for the accurate diagnosis and optimal treatment of pediatric glioma patients.

In summary, the fusions identified here illustrate that a subset of PXA can harbor RTK fusions rather than MAP kinase alterations, which extends the molecular spectrum of this tumor type beyond what is currently recognized [2].
In addition, we highlight the diagnostic challenge in differentiating RTK-fused PXA and IHG given our novel findings that RTK fusion can be seen in both tumor types.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00401-021-02396-y.

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Data availability Raw sequencing and DNA methylation data files are available from the authors upon request.

Declarations

Conflict of interest DAS and AP are members of the editorial board for Acta Neuropathologica. They were not involved in the assessment or decision-making process for this manuscript. The authors declare no competing interests related to this report.

Ethical approval This study was approved by the Committee on Human Research of the University of California, San Francisco, with a waiver of patient consent.

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References

1. Aghajan Y, Levy ML, Malicki DM, Crawford JR (2016) Novel PPP1CB-ALK fusion protein in a high-grade glioma of infancy. BMJ Case Rep. https://doi.org/10.1136/bcr-2016-217189

2. Brat DJ, Ellison DW, Figarella-Branger D, Hawkins C, Louis DN, Ng HK et al (2021) WHO classification of central nervous system tumours, 5th edn. IARC, Lyon

3. Britton HM, Levine AB, Shen Y et al (2021) NTRK2 fusion driven pediatric glioblastoma: identification of oncogenic drivers via integrative genome and transcriptome profiling. Clin Case Rep 9:1472–1477

4. Clarke M, Mackay A, Ismer B et al (2020) Infant high-grade gliomas comprise multiple subgroups. Cancer Discov 10:942–963

5. Guerreiro-Stucklin AS, Ryall S, Fukuoka K et al (2019) Alterations in ALK-ROS1-NTRK-MET drive a group of infantile hemispheric gliomas. Nat Commun 10:4343

6. Jones DT, Hutter B, Jäger N et al (2013) Recurrent somatic alterations of FGFR1 and NTRK2 in pilocytic astrocytoma. Nat Genet 45:927–932

7. Kline CN, Joseph NM, Grenert JP et al (2017) Targeted next-generation sequencing of pediatric neuro-oncology patients improves diagnosis, identifies pathogenic germline mutations, and directs targeted therapy. Neuro Oncol 19:699–709

8. Louis DN, Perry A, Wesseling P et al (2021) The 2021 WHO classification of tumors of the central nervous system. Neuro Oncol 23(8):1231–1251

9. Ng A, Levy ML, Malicki DM, Crawford JR (2019) Unusual high-grade and low-grade glioma in an infant with PPP1CB-ALK gene fusion. BMJ Case Rep. https://doi.org/10.1136/bcr-2018-228248

10. Phillips JJ, Gong H, Chen K et al (2016) Activating NRF1-BRAF and ATG7-RAF1 fusions in anaplastic pleomorphic xanthoastrocytoma. Brain Pathol 29:85–96

11. Phillips JJ, Gong H, Chen K et al (2019) The genetic landscape of anaplastic pleomorphic xanthoastrocytoma. Brain Pathol 29:85–96

12. Wu Z, Abdullaev Z, Pratt D et al (2021) Impact of the methylation classifier and ancillary methods on CNS tumor diagnostic. Neuro Oncol. https://doi.org/10.1093/neuonc/noab227

13. Zhang J, Wu G, Miller CP et al (2013) Whole-genome sequencing identifies genetic alterations in pediatric low-grade gliomas. Nat Genet 45:602–612

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