Comprehensive molecular profiling of paediatric CNS tumours – experience from a single neuropathology centre

Zita Reisz [1], Ross Laxton[1], Leena Bhaw[1], Bassel Zebian[2], Cristina Bleil[2], Keyoumars Ashkan[2], Jozef Jarosz [3], Andrew King [1], Istvan Bodi [1], Safa Al-Sarraj [1]

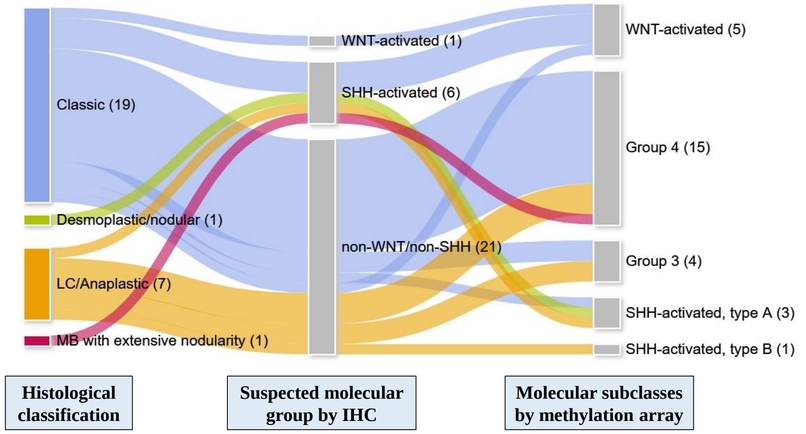
[1] King's College Hospital, NHS Foundation Trust, Department of Clinical Neuropathology, London, United Kingdom, [2] King's College Hospital, NHS Foundation Trust, Neurosurgery, London, United Kingdom, [3] King's College Hospital, NHS Foundation Trust, Department of Radiology, London, United Kingdom

Introduction: There is increasing evidence that molecular profiling of paediatric CNS tumours not only gives a deeper insight in their underlying genomic and epigenetic mechanisms but helps improve the diagnostic practices with more reliable prognostic stratification and identification of new therapeutic targets.

Methods: A retrospective study was performed on 397 paediatric cases diagnosed between 2014 and 2020. Of 283 neuroepithelial paediatric tumours, 130 cases underwent methylation profiling and 46 cases were tested by NGS/RNA fusion panel.

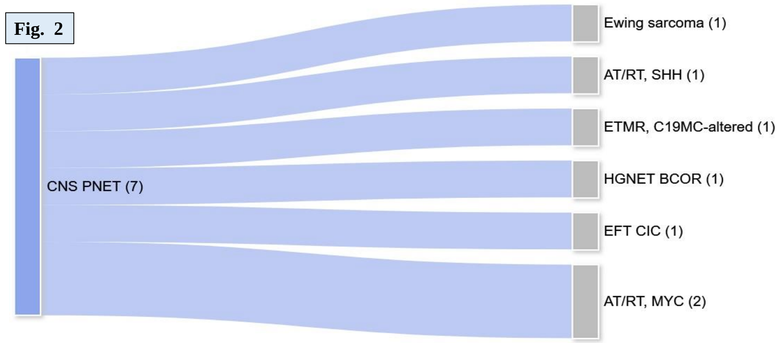
Embryonal tumours: Methylation array succesfully classified 28 medulloblastomas into four main molecular subgroups (Fig.1). The diagnosis was confirmed in 78.6 % of the cases. Clinically meaningful change was found in 6 samples (21.4 %) due to inconclusive stains for surrogate immunohistochemical markers particularly in SHH-activated and non-WNT/non-SHH molecular types. CNV plot analysis revealed MYC or MYCN amplification in 2 non-WNT/non-SHH activated, group 4 medulloblastomas. Next generation sequencing identified pathogenic mutations in 6 tumours including SUFU, PTCH1 and TERT mutations in SHH-activated and CTNNB1 mutation in WNT-activated cases.

Fig. 1



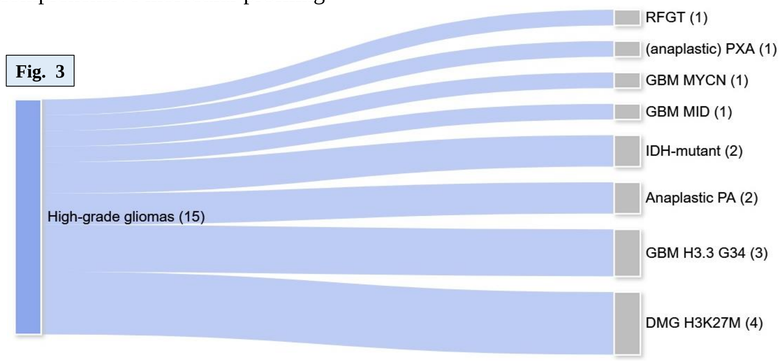
Other non-medulloblastoma type embryonal tumours, formerly called CNS PNET, formed a heterogeneous group of molecularly-defined entities. Among these, embryonal tumour with multilayered rosettes (ETMR) and atypical teratoid/rhabdoid tumour (AT/RT) revealed characteristic histological features and were diagnosed by LIN28A positivity and loss of INI-1 nuclear staining, respectively. However, methylation array was essential to diagnose most of the remaining embryonal tumours with undifferentiated morphology and non-specific immunoprofile (Fig. 2). Multimodal paediatric NGS panel and FISH was an alternative method to detect driver molecular alterations, like BCOR internal tandem duplication, aberrant EWSR1 fusion transcript and EWSR1 gene rearrangement.

Fig. 2



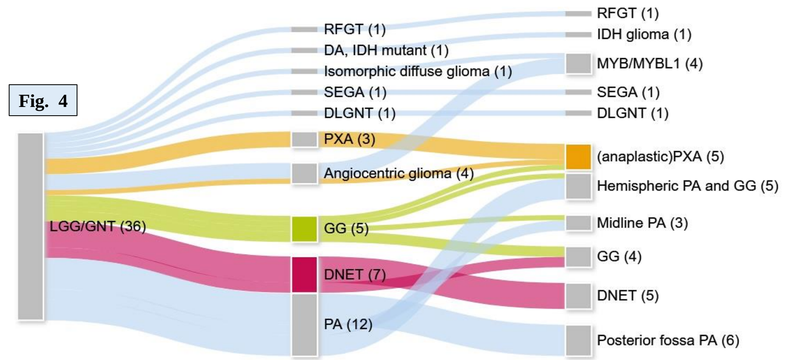
High-grade gliomas: The most common high-grade paediatric/young adult gliomas (HGG) in our cohort were the diffuse midline glioma, H3K27M mutant (12 cases) and glioblastoma, H3.3 G34 mutant (4 cases) of a total 40 cases. These tumours revealed characteristic clinical presentation (location, age) and they strongly expressed the surrogate immunohistochemical markers. Methylation array confirmed the diagnosis in 7 of these cases. Further common HGG subtypes were anaplastic pilocytic astrocytomas, anaplastic pleomorphic xanthoastrocytomas (PXA) and IDH-mutant (adult-type) HGG-s, all were recognized by methylation array (5 cases) (Fig.3). There was one case with histologically suspected high-grade features, reclassified as rosette-forming glioneuronal tumour (RFGT). Diagnosis of remaining cases often with heterogeneous morphological patterns and unusual clinical settings, however, was very difficult and required extensive molecular investigation. Many of these cases revealed no match by methylation array (7 cases) and required to be investigated by NGS and RNA fusion panel to identify underlying mutations typical of certain recently-described tumour types (TRIM24-MET fusion, MN1-PATZ1 fusion). Diagnosis of some of the HGG cases remained elusive even after comprehensive molecular profiling.

Fig. 3



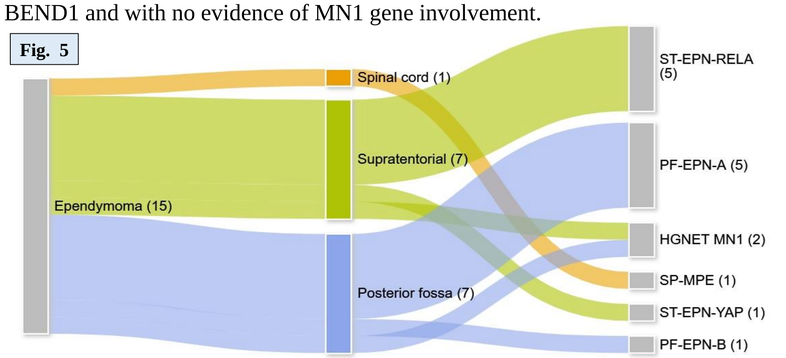
Low-grade gliomas: Low-grade paediatric gliomas (LGG) and glioneuronal tumours (GNT) included several different entities many of them showed overlapping histological features, probably representing a spectrum within MAPK-pathway altered tumours. Methylation profiling was undertaken on 25 LGG-s and 11 GNT-s to achieve a firm diagnosis. The molecular analysis confirmed the histological impression in 75% of the cases and refined 8% of the diagnoses (Fig.4). Significant change was detected in 17% of the samples most frequently involving gangliogliomas (GG), PXA-s and dysembryoplastic neuroepithelial tumours (DNET). KIAA1549-BRAF fusion was identified in 38 pilocytic astrocytomas (PA) and 1 diffuse leptomeningeal glioneuronal tumour (DLGNT), while BRAFV600E mutation was detected in 6 PXA-s, 4 PA-s and 16 GG-s. FGFR1 alteration was seen in 3 DNET-s and 1 RFGNT.

Fig. 4



Ependymomas were subdivided into 9 main molecular subgroups across 3 anatomical compartments. We performed methylation profiling on 15 ependymomas (of a total 27 cases), all successfully classified with a high calibrated score (Fig.5). The diagnosis was confirmed in 80 % of the cases. After inconclusive immunohistochemistry for L1CAM, 1 case was reclassified as ependymoma, YAP fusion. Retrospective histological review found 2 cases with unusual morphological features, immunoprofile and focal astroblastomatous rosettes, both reclassified as HGNET, MN1 altered tumour, causing clinically significant change in the diagnosis (13%). Two supratentorial ependymomas were also tested by RNA fusion panel, confirming diagnostic fusions for C11orf95-RELA and YAP1-MAMLD1. Interestingly, both astroblastomas revealed unusual fusion partners for EWSR1-BEND1 and with no evidence of MN1 gene involvement.

Fig. 5



Conclusion: Implementation of molecular techniques in diagnostics of paediatric brain tumours is crucial to achieve a firm diagnosis, particularly in cases with unusual morphology or clinical behaviour. Nevertheless, even after using these techniques the diagnosis of some cases can still remain elusive.