diagnosis, providing both subgroup assignment and genome wide DNA copy number profiles. These latter data can be used to identify intragenic breakpoints which are frequently associated with structural variations resulting in therapeutically targetable oncogenic fusion genes. To systematically assess the landscape of these alterations, we combined publicly available methylation datasets resulting in a total of 5660 CNS tumours, around half paediatric, and including >1000 high grade glioma and DIPG. These were analysed by standard methodology (MNP, conumee), and intragenic breakpoint enrichment was compared within methylation subgroups, superfamilies, and tumours with no high-scoring classification. Benchmarking included sequence-verified cases such as infant hemispheric gliomas (IHG) with *ALK*(15%) and *ROS1*(7%) fusions, and pathognomic alterations associated with specific entities such as RELA-EPN, MYB-LGG and HGNET-MN1. We identified previously unreported enrichments of well-recognised fusion targets such as *NTRK2*in GBM_MID and *NTRK3*in DMG_K27 (both 5%), *MET*in A_IDH / A_IDH_HG (3–5%), and *FGFR1/3*in GBM_G34 (8–9%). Novel recurrent kinase gene candidates to be verified and explored further include *IGF1R*in 2–12% cases spanning glioma subgroups, and *TIE1*in poorly classified tumours. This latter 'NOS' group were also enriched in various transcription factor targets of breakpoints, including *TCF4*and *PLAGL2*. Despite limitations due to sample quality, resolution or balanced translocations, breakpoint analysis of methylation copy number profiles provides simple screening for structural rearrangements which may directly influence targeted therapy in paediatric CNS tumours.

PATH-18. HIGH-GRADE NEUROEPITHELIAL TUMOR (HGNET) IN A PEDIATRIC CASE-SERIES

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The central nervous system (CNS) high-grade neuroepithelial tumor is a recently described molecular entity. We report 2 new CNS HGNET cases sharing common clinical presentation and pathologic features. In summary, CNS HGNET represents a rare tumor occurring in young patients with dismal prognosis. We think it is important to report these cases to spread the experience and raise the knowledge of the medical community.

PATH-19. MOLECULAR CLASSIFICATION BASED ON THE DNA METHYLATION PROFILE OF CENTRAL NERVOUS SYSTEM (CNS) TUMORS IN CHILDREN: TWO-YEARS EXPERIENCE AT THE BAMBINO GESÙ HOSPITAL

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INTRODUCTION: Pediatric brain tumors (PBT) represent the second most common pediatric cancer, with the highest mortality rate among childhood malignancies. Improvement of PBT diagnostic accuracy is fundamental to optimize treatment strategy. OBJECTIVES: We aimed to explore the impact of DNA methylation arrays implementation in PBT clinical practice. METHODS: 214 PBT were analyzed by Illumina 850KEPICmethylation array. Low score and discordant cases were collegially reviewed. RESULTS: Calibrated score was 0.8 or higher in 159 cases (74.3%), with pathological diagnosis confirmation in 132 cases and molecular subgroup definition in 47 of them, including cases of medulloblastoma, CNS neuroblastoma FOXR2, HGNET MN1; methylation profiling amended diagnosis in 10 cases, e.g. HGNET BCOR and anaplastic PXA, was non-contributory in 4 and misleading in 12 cases, including glioneural tumors and tumors arising in syndromic contexts. Calibrated score ranged between 0.8 and 0.3 in 37 cases (17.3%) and was below 0,3 (no match) in 18 cases (8.4%). Calibrated score below 0,8 was more frequently assigned to low grade gliomas and low grade glioneural tumors ($p < 0.0006$). Challenging/very rare cases, e.g. intracranial AFH with EWSR1:CREM fusion and nonRELA supratentorial ependymomas, were assigned to "no match subgroup"; in syndromic patients the score tended to be lower ($p=0.07$); no correlation between score and age < 3-years was found ($p=0.1$). CONCLUSION: Methylation profiling refine on diagnostic accuracy in PBT classification. Improvements are needed in classifying low grade glioma/glioneuronal tumors and challenging/very rare PBT. In syndromic cases, there is a high rate of misleading profiles and/or low scores.

PATH-20. METHYLATION ARRAY PROFILING OF PEDIATRIC BRAIN TUMORS; SINGLE CENTRE EXPERIENCE

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BACKGROUND: Significant heterogeneity of pediatric brain tumors poses major challenge on diagnostics. Therefore, we aimed to evaluate feasibility of methylation array in the diagnostic process. METHODS: Methylation array (Infinium MethylationEPIC, Illumina) was performed on DNA extracted from fresh frozen tissue from prospective newly diagnosed and selected retrospective patients. Results from Heidelberg classifier (www.molecularneuropathology.org) were compared to the histological diagnosis and further genetic testing was performed to establish integrated morphological/molecular diagnosis. RESULTS: Within years 2018–2019, we performed methylation array profiling of 102 samples consisting mainly of ependymoma, medulloblastoma high-grade and low-grade glioma. High calibrated score (>0.9) was achieved in 62 patients (61%). In 46 cases (74%) with score >0.9, the histological diagnosis matched the methylation class (MC). In the remaining cases (16) that were classified by histopathology mainly as ependymomas, the methylation profiles were classified as novel molecular entities (HGNET_BCOR, HGNET_MN1, etc.) or different tumor type. In 40 cases (39%) with the score <0.9, six were found to have high normal tissue content. Nine cases had no match in the classifier and 25 were assigned MC with score 0.3 to 0.89. In 20 out of 34 cases with low score, the molecular diagnosis could be confirmed based on copy number variants inferred from the methylation array or using additional testing for gene fusions and mutations. CONCLUSIONS: Our experience on the first 100+ cases demonstrated that methylation array could be integral part of diagnostic process in order to establish integrated morphological and molecular diagnosis of pediatric brain tumors.

PATH-21. TELOMERE LENGTH ANALYSIS OF CNS TUMORS IN THE PEDIATRIC BRAIN TUMOR ATLAS

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Subsets of pediatric cancers, including high grade glioma (pHGG), have high rates of uniquely long telomeres, associated with ATRX gene mutations and alternative lengthening of telomeres (ALT). Ultimately, these cancers may benefit from a therapy stratification approach. In order to identify and further characterize pediatric brain tumors with telomere lengthening (TL), we determined the intratelomeric content *in silico* from paired WGS of 918 tumors from CBTTC Pediatric Brain Tumor Atlas (PBTA). The results were highly concordant with experimental assays to determine ALT in a subset of 45 pHGG tumors from the set. Overall, 13% of the PBTA cohort had telomere lengthening. We confirmed the highest rate of TL (37%) in the pHGG cohort (37/100 tumors; 30/82 patients). There was no statistical difference in age, gender or survival in subset analysis. As expected, the patient pHGG tumors with telomere lengthening were enriched for ATRX mutations (60%, q= 1.76e-3). However, 6 tumors without ATRX mutation also had normal protein expression, suggesting a different mechanism of inactivation or TL. The pHGG tumors with telomere lengthening had increased mutational burden (q=8.98e-3) and included all known pHGG cases (n=6) in the cohort with replication repair deficiencies. Of interest, the second highest rate of telomere lengthening was 9 subjects (24%) in the craniopharyngioma cohort. None of the craniopharyngioma tumors had ATRX mutations or low ATRX expression, and 55% of those with TL had CTNNB1 mutations. Finally, lower rates of telomere lengthening were found in medulloblastoma (10%), ependymoma (10%), low grade astrocytoma (8%) and ganglioglioma (7/47, 15%).

PATH-22. COMPARISON OF SUPERVISED CLASSIFICATION METHODS FOR CENTRAL NERVOUS SYSTEM TUMORS BASED ON DNA-METHYLATION

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Classification of brain tumors using methylation profiling is an important diagnostic advance, reducing subjectivity and improving interpretability of clinical outcome data. Despite the recognized value of methylation profiling