**The impact of molecular profiling on brain tumour diagnosis and treatment: A paediatric population-based study**

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*Abbreviations*: CNS central nervous system; ETMR embryonal tumour with multi-layered rosettes; FISH fluorescence *in situ* hybridisation; FFPE formalin-fixed paraffin-embedded; HGNET high grade neuroepithelial tumour, IHC immunohistochemistry; NOS not otherwise specified; PNET primitive neuro-ectodermal tumour; WHO World Health Organisation.

**Abstract**

The application of routine genomic profiling in tumour diagnosis poses significant challenges. There is marked variation in the implementation of genomic data, while optimal integration with conventional diagnostic technology remains uncertain despite several studies reporting improved diagnostic accuracy and selection for targeted treatments and stratification for trials. Many earlier findings are based on research cohorts or specific tumour groups, often measuring the impact of treatment only with respect to experimental therapies. There is a need for the evaluation of the added value in routine clinical practice and impact on conventional, as well as experimental, treatments. To address these questions, we have assessed the routine implementation of genomic profiling (by a DNA methylation-based approach) in childhood brain tumours using two large national cohorts. The first includes routinely diagnosed childhood brain tumours in the UK (n=306). Molecular profiling added a unique contribution to diagnosis in 34% of cases in routine diagnostic practice, and we estimated that it could change conventional treatment in 3.5% of patients. In the second cohort we enriched for cases that historically had been diagnostically difficult (n=203). In these cases 49.7% could be diagnosed using standard methods, with the addition of methylation profiling solving a further 17.2% of cases. The remaining 30.1% cases remained unresolved despite specialist pathology and genomic profiling. Together these data provide estimates of impact that could be expected from routine implementation of genomic profiling into clinical practice, and also indicates limitations where additional techniques will be required.

**INTRODUCTION**
The incorporation of complex molecular pathology into routine clinical practice offers enormous potential to improve diagnostic accuracy and tailor treatment stratification, but it also poses significant challenges [1, 2]. Not only are there practical limiting factors such as cost, access and accreditation, but it is also unclear whether we can extrapolate the impact from carefully curated research cohorts to routine clinical practice. In particular, genome-wide profiling studies have primarily taken place within research environments or as part of clinical trials [3, 4]. Their implementation in routine clinical practice is at best variable and at worst, controversial.

The accessibility of genomic data is of particular importance for the diagnosis of paediatric brain tumours for two main reasons. The first is that a significant proportion of tumours of the central nervous system (CNS) have been re-classified into distinct tumour subgroups based on their distinct molecular profiles [5-8]. Secondly, due to diagnostic challenges in many of the tumours, genomic data has a significant impact on the therapies offered to children; many of whom are at a high-risk of death or long-standing disability depending at least in part on the treatment regime.

A recent description of a methylation classifier for CNS tumours holds promise as an adjunct to conventional diagnostic practices[9, 10]. The methylome is thought to reflect the cell of origin and is maintained during tumour evolution, suggesting that tumours of the same molecular subgroup have consistent methylation profiles [11]. Using methylation array data, the authors developed a model that predicts a tumour’s molecular subtype based on a large reference cohort of adult and paediatric CNS tumours [9]. Internal validation suggested good predictive power, with a reported diagnostic concordance of up to 88% of cases.

To reflect the differences between adult and paediatric CNS tumours, the classifier model was developed using a large reference cohort containing over 40% of samples from patients under the age of 19 years[9]. Indeed, many of the methylation classifier’s predicted classes and subclasses are associated with specific age-groups, particularly in children. However, the number of paediatric cases within the author’s internal and external validation cohorts under-represented.

Since 2016, Great Ormond Street Hospital (GOSH) has undertaken DNA methylation arrays alongside histopathology assessment and routine molecular testing. As a large paediatric specialist tertiary centre, we receive many complex referral cases from across the UK. We aimed to assess the impact of routine molecular profiling in clinical practice and estimate the added value on diagnosis in the context of modern standard-of-care pathology. Importantly, we set out to determine how often refinement of diagnosis would change treatment, considering both conventional and experimental trial-based regimes. To this effect, we have assessed the impact of DNA methylation profiling on the routine diagnosis of childhood brain tumours in a real-time clinical context.

Our experience is an exemplar of how genomic approaches can be effectively integrated into clinical practice, and how analysis usually confined to research can be reliably reported and translated into clinically meaningful data.

**Methods**

**Sample inclusion criteria**

Our data includes two national UK cohorts: a diagnostic cohort of 306 samples to measure the impact on routine diagnosis and a second archival cohort encompassing 203 diagnostically unresolved cases.

Diagnostic Cohort: We included cases spanning a 24-month period (Sept 2016- Sept 2018), limited to children and young adults under the age of 19 years, where a DNA methylation array was performed at GOSH in real-time for clinical purposes. The decision to perform an array was at the discretion of the neuropathologist; typically most primary high grade tumours would be profiled (see results section). Local and referred cases were included, with a small number (n=19) received from centres from outside of the UK. Cases undertaken for research purposes were excluded.

Archival cohort: We searched for UK-based cases of high-grade CNS paediatric tumours (grades III and IV) reported between 1990 and 2018, that either failed to achieve a confident diagnosis at the time of initial diagnosis or fell into groups now recognized to represent a mixture of novel molecularly-defined tumour groups (e.g. CNS PNETs/supratentorial PNETs). We excluded cases with a well-defined and definitive initial diagnosis including: medulloblastoma, glioblastoma, ependymoma, AT/RT, Ewing’s sarcoma, teratoma and sarcoma.

**DNA preparation and bisulphite conversion**

DNA was extracted from a total of 50 µm formalin-fixed paraffin-embedded (FFPE) tissue taken either as 5x10 µm rolls or macro-dissected sections to enrich for tumour cell content. In cases where multiple blocks were present, regions with the most representative histology, overall tumour cell content and tissue viability were selected. DNA was extracted using the Promega Maxwell 16 FFPE Tissue LEV DNA Purification Kit and quantified using a Nanodrop, before bisulphite conversion of up to 500 ng DNA, using the Zymo EZ DNA Methylation-Gold kit. Bisulphite converted FFPE DNA was then treated with the Illumina Infinium FFPE DNA Restore kit.

**850K arrays**

Bisulphite converted and restored DNA was assayed using Illumina Infinium MethylationEPIC BeadChip arrays, according to the Infinium HD FFPE Methylation Assay automated protocol (Illumina).

**Methylation classification**

Methylation data was imported into R v3.5.3 using minfi[12] (version 1.22.1) and normalised with the included function *preprocessIllumina*. Missing CpG beta values were imputed using the *impute.knn* function implemented in the impute package[13]. A DNA methylation classification model (MNP v11b2, current at the time of implementation) was used to assess the methylation profile of each tumour. This tool was developed for research and is freely available online (www.molecularneuropathology.org/mnp) [9]. Sample methylation data were compared to a reference cohort of 2,801 CNS tumours across 91 tumour types, resulting in an output indicating the best match of tumour diagnosis and a corresponding calibrated score[9]. Scores range from 0-1, and scores of ≥0.9 were used for robust classification during the period of the study. After classification, the predicted tumour class with the highest score was recorded alongside the calibrated score.

**Reporting of methylation outputs**

When the classifier’s predicted output gave a calibrated score ≥0.9 the predicted methylation class was used in the clinical report. When scores were <0.9, the classifier was deemed to have failed and the prediction was not included in the clinical report[10]. All outputs were considered alongside our standard neuropathology workup[14, 15] and when relevant, further diagnostic testing was performed to confirm the result. Retrospectively, the impact of each prediction and calibrated score was compared to the final reported diagnosis.

**Copy number plots**

Copy number (CN) plots were generated from methylation data using the R package conumee[16]. These plots were attached to the clinical reports, however during the timeframe of this study the interpretation of CN plots was not routinely reported. For the purposes of this study, CN plots were reviewed independently of the clinical reports and used to identify diagnostically relevant CN changes in specific tumour types. Gains and losses were called when probe intensity was ±0.15 on log2 scale and amplifications were called when signals were >0.6[17, 18].

**Assessment of diagnostic impact**

The rationale for undertaking methylation analysis in clinical cases was categorised as: (i) tumours that were difficult to diagnose, (ii) rare tumour types requiring further confirmation, (iii) cases where the purpose of the array was to determine the subtype of a tumour (e.g. ependymoma, medulloblastoma) and (iv) cases with limited available diagnostic material.

The impact on the final diagnosis in clinical cases of the predicted methylation class was divided into 5 categories: (i) classification that confirmed the final diagnosis but did not add additional information, (ii) classification that confirmed and refined the final diagnosis by providing additional molecular subtyping not available by histopathological evaluation alone, (iii) classification that amended the initial diagnosis, leading to a change in final diagnosis, (iv) classifications of uncertain significance and (v) classifications considered potentially misleading in the context of other diagnostic findings.

**Assessment of clinical impact**

Methylation data from the clinical cohort which refined or altered the final diagnosis were retrospectively assessed by a senior paediatric neuro-oncologist (DH) to determine if the amended or refined diagnosis would indicate a change in therapeutic strategy according to relevant national guidance for childhood tumours in the UK at the time of diagnosis. We considered methylation data to have had a clinical impact when the patient’s treatment would have differed if the data had not been available. We also identified cases where the methylation data could in the future help to triage patients that might be eligible for forthcoming trials; such as targeted therapies or improved risk stratification that would influence trial eligibility (e.g. in medulloblastoma). We analysed the latter data separately as it represents a more speculative component.

**Pathology review of archival samples**

A panel of immunohistochemistry regularly assayed as part of our current diagnostic workup for embryonal tumours was performed on a total of 203 tumour cases from the archival cohort. This included: GFAP, synaptophysin, Ki-67, NeuN, OLIG2, INI-1, SMARCA4, LIN28A, mutant H3K27M, H3K27 trimethylation, CD56, CD99, EMA, p65/RELA and L1-CAM [19]. FFPE sections were cut at 3 µm and staining was carried out on a LEICA BondMax automated stained. The histopathology was then reviewed by an experienced paediatric neuropathologist (TSJ) to determine if a confident diagnosis could be offered on the basis of the neuropathological features. DNA methylation arrays and the classifier algorithm were performed as described above using MNP v2 and v4. Where appropriate, cytogenetic testing (fluorescence in-situ hybridisation, FISH) and confirmation of suspected mutations (sequencing, RT-PCR) was performed to complete the final reviewed diagnosis.

**Mapping archival samples against reference cohorts**

Sample methylation data were read into R using Minfi and preprocessed using the included preprocessIllumina function. Beta values were extracted and matched against those in the MNP reference cohort dataset (v4)[9]. These combined data were used to generate t-SNE plots containing both reference and GOSH cohorts for manual inspection of clustering in cases where GOSH samples could not be robustly classified by the MNP algorithm. Research samples whose co-ordinates were within or directly adjacent to methylation reference subgroups were considered to belong to the respective tumour subgroup.

**Ethical approval**

Approval of review of the clinical cohort was given by Great Ormond Street NHS Foundation Trust as service evaluation (Registration No. 2301). The archival cohort was analysed under ethical approval granted by BRAIN UK tissue bank (REC: 14/SC/0098, Reference 16/007)

**Results**

**Implementation of methylation profiling in routine clinical practice**

To determine the impact of molecular testing on diagnostic yield we reviewed the GOSH experience of methylation profiling in childhood CNS tumours over a 2-year period. Specifically, we diagnosed 484 brain tumours in patients under 19 years of age, compared to an estimated brain tumour incidence in this age group of 633 cases per year[20]. This cohort therefore approximately represents 40% of the UK’s CNS childhood tumours (total national population of 66 million [21]). We undertook DNA methylation arrays for diagnostic purposes in 306 cases or 63.2% of our total cases reported; the annual equivalent of almost one quarter (24.2%) of the population’s childhood brain tumours (Figure 1).

The cases included in this cohort were those that were spontaneously referred by local neuropathologists for DNA methylation profiling for diagnostic reasons, and the majority of high-grade tumours were profiled (Figure 1D). To determine the effect on diagnosis and treatment of children’s brain tumours, we categorised cases according to the primary indication for undertaking an array (Supplementary figure 1): 162 cases were requested specifically to establish a tumour subtype (52.9% of cases) e.g. in medulloblastoma or ependymoma; 115 cases were undertaken as they were diagnostically difficult cases (37.6%); 19 were undertaken where the diagnosis was certain but the tumour type was considered unusual in the age group (6.2%); and 10 were undertaken to improve diagnostic uncertainty when the biopsy was small (3.3%).

**Diagnostic yield of clinical methylation profiling**

We analysed three aspects of the molecular data from methylation arrays. The principal outcome was classification using an algorithm from the DKFZ [9]. In keeping with previous publications[9, 10], we regarded a calibrated score greater than 0.9 as successful. From the entire diagnostic cohort (irrespective of DNA quality), 149 cases (48.7%) gave a calibrated score of 0.9 or greater. Diagnostic impact was assessed by reviewing the reports to determine what effect the array had on the real-time diagnostic process (see methods, Figure 2) [9, 22].

Cases where the array matched and therefore confirmed the final diagnosis accounted for 26.2 % of classifying cases (12.7% of all cases, 39 cases). Diagnoses in this group mostly belonged to CNS tumours with molecular subgroups that were identifiable by existing and well-established diagnostic tests. These included WNT-activated medulloblastoma, diagnosable by nuclear beta-catenin immunopositivity and sequencing of the *CTNNB1* gene; diagnosis of ependymoma-RELA altered, identifiable by nuclear p65/RELA and/or L1-CAM immunopositivity; and diagnosis of diffuse midline glioma with a histone mutation, identifiable by nuclear H3 K27M immunopositivity and sequencing of the most commonly altered histone genes (*H3F3A, HIST1H3B* and *HIST1H3C*).

Array predictions that both confirmed and refined the final diagnosis by providing additional molecular subtyping data, not otherwise available by existing histopathological or molecular evaluation, accounted for 64.4 % of the classifying cases (32.4% of all cases, 99 cases). Many of these tumours were those where an array was used as the primary method of subtyping e.g. many of the molecular subtypes of medulloblastoma, ependymoma (posterior fossa subtype A, PFA) and some classes of high-grade glioma (e.g. MYCN and RTKIII subtypes).

There were 5 array predictions which led to an amended final diagnosis (Supplementary table 1A). Three of these belonged to two newly recognized molecular groups, high grade neuroepithelial tumour (HGNET) with MN1 alteration and HGNET with BCOR alteration [23].

In no cases was the array entirely misleading if taken in the context of other radiological and pathological data. We only considered 3 array predictions to be potentially misleading if interpreted in isolation (Supplementary table 1B). In these instances the clinical management was not likely to have changed. The first case was an embryonal tumour that classified as a pineoblastoma however, on review there was no radiological involvement of the pineal gland. It is of course possible that this is a result of the limitation of the radiology rather than the array, or it may represent an ectopic origin for a pineoblastoma[10]. In two cases, the classifier result reported control brain tissue but on review, these cases had relatively low tumour content.

In a further 3 cases, the array made a prediction that was considered potentially significant, but it was not possible to confirm this finding in a clinical or histological context, and we regarded these results as of uncertain significance (Supplementary table 1C).

**Factors that affected diagnostic yield**

To explore the factors that affected the score, we reviewed potential confounding factors including technical factors (hybridisation success, DNA source), clinical indication for the array, patient age and diagnosis.

All cases were processed on the array irrespective of the DNA quality. Therefore, to analyse the impact of technical factors (such as DNA quality) on the diagnostic yield, we analysed the percentage of probes that failed and compared this to the calibrated score from the classifier. We found that there was correlation between probe failure rate and the success of the classifier. None of the 20 (6.5%) cases with a probe failure rate of more than 5% gave a calibrated score of more than 0.9. There were 12 (3.9%) cases with a probe failure rate of 2-5% and most of these cases (10) did not classify. There were 22 (7.2%) cases with a probe failure rate of 1-2% and most of these (19) classified. Therefore, high probe failure rates do predict a poor classification score, with the majority of cases failing to classify where the probe failure rate was over 2% (Figure 3). Overall, 1.3% and 19.1% of the all the cases that did and did not classify respectively, had probe failure rates above 2%. This gives an approximate estimate of the impact of technical failures on the diagnostic utility of the arrays.

Next, we hypothesised that the classifier results may vary depending on the different clinical contexts. To address this, we analysed the diagnostic yield based on the patient’s age, site of preparation, final diagnosis and the clinical indication for the array. We found no correlation between age and diagnostic yield suggesting that the classifier worked equally well across all ages of children and young adults (Supplementary figure 2).However,the rate of diagnostic yield depended on the clinical indication to undertake an array (Figure 3). The highest yields were from cases undertaken specifically for sub-typing or confirmation of secure diagnoses. There was intermediate yield when the arrays were undertaken to solve diagnostically challenging cases and the lowest diagnostic yield occurred from cases with small biopsies. Our findings suggest that the expected success of methylation analysis depends on the clinical question being addressed by the array.

To determine if the performance of the classifier differed in different tumour types, we considered how the calibrated scores varied according to the final diagnosis (Figure 2C). The tumour type with the highest success rate was medulloblastoma in which 59 of the 79 cases profiled (74.7%) produced a confident subtype. Ependymoma had more modest results, where 15 of the 29 cases (51.7%) classified. Although notably most RELA-altered ependymomas (7 out of 9 cases) successfully classified by array.

Fifty-nine patients were diagnosed with a high-grade astrocytoma (grades III and IV) and of these, 40.7% (n=24) were classifiable (Figure 2C). However, the rate was higher in specific subtypes. For example, diffuse midline gliomas with a histone H3K27 mutation achieved a confident score in 7 out of 10 cases. Glioblastomas carrying a histone G34 mutation gave a confident calibrated score in all 4 cases. An increase in grade was observed in 10 high-grade astrocytoma cases to glioblastoma by the addition of methylation data.

Of the 85 patients with a final diagnosis of a low-grade glioma or glioneuronal tumour, a confident calibrated score ≥0.9 was assigned in only 32.9% of cases (n=28), compared to our cohort’s average of 49% (Figure 2C). This suggests that this tumour group remains particularly challenging to classify by this technique.

**Copy number data**

Inferred copy number (CN) data generated from methylation arrays were not included in the clinical reports because they were not accredited during the time of this study. Therefore to assess the potential of CN data in our clinical setting, we retrospectively compared the CN results to existing diagnostic molecular data (i.e. FISH) in medulloblastoma and embryonal tumours with multi-layered rosettes (ETMR).

*MYC* and *MYCN* amplification are poor prognostic factors in medulloblastoma[24]. Both FISH and CN plots were available for 62 out of 79 reported medulloblastomas, with a total of 12 patients reported to have amplifications in either *MYC* (n=6) or *MYCN* (n=7) identified by FISH (one patient had the unusual combination of both) (Figure 4). The CN plots had a specificity of 100% for both *MYC* and *MYCN* amplification when compared to FISH. However, the sensitivity of the array was much lower at 67% for *MYC* and 57% for *MYCN* when compared to FISH. It is likely that the lower sensitivity is due to the difficulty in detecting focal amplifications by bulk DNA techniques such as the array.

Next, we considered the detection of amplification of the microRNA cluster on chromosome 19 (*C19MC*) that defines *C19MC*-altered ETMRs. In all four ETMRs in the series, *C19MC* amplification was detected by both FISH and by array CN plot analysis (data not shown).

***MGMT* promoter methylation assessment in paediatric cases**

*MGMT* promoter methylation is considered of prognostic relevance in adult gliomas and associated with temozolomide sensitivity [25]. However, there are few studies addressing it’s utility in childhood cancers[26, 27] and its prognostic utility in paediatric glioma remains unconfirmed[28, 29]. Inferred methylation status from our array data predicted that 94% of our paediatric cohort had unmethylated *MGMT* promoters. Only 9 patients (2.9%) were predicted to have a methylated *MGMT* promoter (with a high confidence interval and low probe rate failure): 8 cases were high grade tumours (grade III and IV) and 4 of these were glioblastoma (grade IV). A fully integrated diagnosis could be achieved in 6 of the 9 cases, which were 2 ependymoma *RELA*-altered (2 out of 9 diagnosis of ependymoma *RELA*-altered), 2 ETMR *C19MC*-altered (2/4) and 2 Glioblastoma G34-mutant (2/4). As previously described no *MGMT* promoter methylation was observed in diffuse midline gliomas with mutant H3K27 [30]. Our data suggest that *MGMT* promoter methylation is rare in children and possibly restricted to small subsets of tumour types.

**Impact on therapy**

We reviewed all cases where the methylation array changed the diagnosis in any way to determine if the additional molecular data would have implications for treatment according to standard treatment protocols used in the UK. We found that in 11 cases, the diagnostic modification would have mandated a change in treatment based on the diagnosis using current protocols (Figure 5). Examples included a change in diagnosis from CNS PNET to RELA-altered ependymoma, ependymoma to high grade glioma (K27M), poorly differentiated tumour to HGNET with MN1 alteration or refinement in medulloblastoma subtypes. To estimate the potential clinical impact of these changes in clinical practice, we expressed this as a percentage of all arrays performed (irrespective of technical failures), indicating that 3.6% of cases tested would have an impact on standard treatment.

We also made an estimate of which diagnoses would predict treatment changes that could be offered in the next few years based on current trial protocols and forthcoming trials. This included identifying new brain tumour subtypes that may harbour targetable aberrations such as infantile hemispheric gliomas which have been recently characterised to harbour gene fusions such as *ALK* or *TRK,* with potential specific inhibitors. In addition, methylation subtyping identified subgroups such as glioblastoma RTK III which in the future could be used to enrich future tyrosine kinase targeting therapeutic trials (Figure 5). When considering these patients, a further expected future change in therapy is suggested for 5.9% of all cases (n=18); approximately bringing the total estimated upper limit on future therapeutic impact to 9.5% of cases tested.

**Impact of suboptimal classification scores**

A calibrated score ≥0.9 is considered to be a robust indicator of CNS tumour classification. At the time of the diagnosis, only scores ≥0.9 were reported in our clinical practice. However lower scores have been suggested to be useful when used with caution[10] and are regularly reported at other centres[22]. As these outputs were not used for real-time diagnosis, we categorised them according to how the classifier’s prediction related to the final diagnosis (Figure 6A). Of the 157 cases with scores below 0.9, there were 18 outputs that matched the final reported diagnosis (11.5%) and 84 outputs with plausible but otherwise unconfirmed molecular subtypes (53.5%). A total of 55 outputs were considered misleading (35.0%), the most common of these being the methylation class meningioma, or paediatric choroid plexus tumour subtype B. Considering all suboptimal scoring cases, 1 in 3 predictions will be misleading. However, if considering suboptimal scores between 0.7 and 0.9, 1 in every 14 predictions was misleading; compared to scores above 0.9 where potentially 1 in every 50 predictions were misleading (Fig. 6B). The lower the calibrated score, the greater the risk of a potentially misleading result (Fig. 6C). We conclude that cases with low calibrated scores should be considered with caution, and always interpreted alongside supporting data from diagnostic workup (IHC, sequencing etc.).

**The impact of molecular profiling in diagnostically challenging cases**

The data in the first cohort give an estimate of the impact of methylation profiling in routine clinical practice of childhood CNS tumours. The data suggested that the techniques was most successful in well-defined tumour entities but showed more variable results where the diagnosis was uncertain. To address this directly, we determined the impact on tumours that have traditionally defied diagnosis. We hypothesised that a proportion of such tumours might be diagnosable by taking advantage of current histological criteria without molecular technology, while others may be solved by the use of methylation profiling and finally, some may remain unresolved despite contemporary histology and molecular analysis.

To estimate the proportion of these cases, we collated a national cohort of archival high-grade brain tumours that either failed to achieve a confident diagnosis at the time of the initial pathological diagnosis or fell into groups that are now recognised to represent a mixed group of molecularly defined entities (e.g. CNS PNETs/supratentorial PNETs). Retrospective cases with sufficient tissue (n=203) were subjected to a standardised immunohistochemistry panel and the histological features were reviewed by a reference neuropathologist (TSJ). In addition, most cases underwent methylation profiling.

Based on the histological features, we were able to offer a specific diagnosis with confidence (i.e. cases where the immunohistochemistry gave an unambiguous results such as INI1 loss in AT/RT) in 67 cases (39.4% of the cohort), suggesting that a high proportion of cases can be resolved without resorting to further molecular testing (Figure 7A and B). In a further 40 cases (23.5%), we were able to achieve a confident diagnosis using methylation profiling in combination with the histology (Figure 7A and C). The remaining 63 cases (37.1%) could not be confidently resolved using the array or histopathology. On further review, we could explain the failure to diagnose 15 of these cases for technical reasons (12 cases had high probe rate failures and poor array quality and 3 cases had low tumour content). One single case was resolved by performing the latest version of the methylation classifier (mnp.v4) and was reclassified as an infant glioma (IHG). The remaining 45 unsolvable cases are likely to be a combination of cases that are novel entities and cases where the technology is not optimal for their identification (Figure 7A).

To further address the unsolvable cases, we plotted these samples against the original DKFZ reference cohort (Figure 7D). From the t-SNE plot we assessed the proximity of unsolvable cases to known methylation classes. We estimate that a further 35% are examples of known entities already part of the existing methylation classifier (e.g. samples that clustered with CNS NB FOXR2). We noted a handful of unsolved cases that clustered together and separately from DFKZ reference groups which warrant further investigation and molecular profiling (Figure 7D). These cases are likely to be rare variants of known CNS tumours described in the literature, for example ETMR-like tumours with *DICER1* mutations[31] or potentially novel, undescribed CNS tumour entities.

**Discussion**

Using two national cohorts of paediatric CNS tumours, we have evaluated the impact and diagnostic limitations of incorporating DNA methylation arrays into routine diagnostic practice. We found that in clinical practice, methylation profiling was likely to alter the diagnosis in 34% of cases and could affect treatment in 11 patients (3.6% of cases).

In assessing the clinical impact of routine use of DNA methylation arrays, we have tried to be conservative by measuring the added value of these techniques beyond standard-of-care testing. Specifically, we have required that an array was necessary for the diagnosis (i.e. the diagnosis had not been achieved by an alternative technique) and that any change in diagnosis would have directed a change in treatment using current UK national protocols (change in regime, escalation or reduction of treatment). Furthermore, in assessing the impact of the array, we have included all arrays irrespective of whether or not they gave an interpretable result. We suggest that these are acceptable approaches for measuring the realistic clinical impact of molecular profiling on clinical practice, avoiding unbiased expectations from clinical teams following implementation of new techniques.

Despite a conservative approach the data supports the routine use of methylation profiling for childhood brain tumours. Taking into account the debilitating long term clinical impact of cancer treatment on the developing brain and the poor prognosis of many childhood brain tumours, we argue that changing treatment protocol in as few as 3-4% of children is likely to be of high significance. Based on this result, and contrary to adult practice[22], our current paediatric practice is to now methylation profile all cases where possible.

The existing literature suggests that there is a variable range of successful classification rates in CNS tumours, and we expected that this depends on the clinical cohort to which the methylation classifier is applied. In our uniquely paediatric cohort we observed a significant proportion of unclassified cases (51%), explained by the high number of complex diagnostic cases and reflecting in part the nature of childhood CNS pathology. The DKFZ validation cohorts report variable rates of classification across 5 external centres (no tumour match in 5-42% of cases, average 22%)[9]. One adult study reported 44% of tested CNS tumours were unclassifiable using a calibrated threshold of 0.84 (Youlden index)[22].

We therefore explored the variability of the data within our paediatric cohort. Our data suggest that as well as technical factors (e.g. poor DNA hybridisation and small biopsies), clinical factors such as the clinical question and the tumour type are important predictors in confident classification. This is important as the *a priori* likelihood of getting a clinically meaningful answer depends on the question that is being asked. For example, if the purpose of the array is to subtype a medulloblastoma, we would expect a high success rate. If in contrast, an array is used to solve a difficult case that has defied conventional diagnostic approaches, it is much less likely to succeed. Low classification rates may also be attributed to poor sample quality and low tumour purity, the latter is likely to be of particular relevance in low-grade infiltrating tumours. In the second cohort we identified a mixed group of cases that all had historically defied diagnosis or they had diagnoses that were historical (e.g. supratentorial PNET). In this group, we were able to offer a confident diagnosis in nearly 40% of cases using histological techniques alone without an array. In part, this reflects an increased recognition of specific entities (e.g. diffuse midline glioma, H3K27 mutant) and the availability of more specific diagnostic markers (e.g. antibodies against mutant H3K27). However, it emphasises that new diagnostic techniques should not be directly compared to historical diagnoses.

The second cohort also identified a group of tumours for which we were unable to offer a confident diagnosis based on the immunohistochemical profile or the methylation profile. It is not possible to fully determine the factors that account for these ‘un-diagnosable’ cases but they are likely to be a mixture of novel tumour entities, and recognised tumour types but where the technology did not diagnose them. The latter may be due to technical factors (such as low tumour content) or intrinsic limitations of the diagnostic methods available for that tumour type. However, based on the clustering of the cases (Figure 7) we estimate that approximately 35% of these ‘un-diagnosable’ cases are actually recognised entities. This permits an upper limit to the number of cases in this group which could potentially be novel (65%, 29 cases) and thus warrant further investigation, especially in unsolved cases which clustered together. A key step for future studies will be the clinical, pathological and molecular characterisation of these cases.

We have used the implementation of methylation profiling in childhood brain tumours to develop an approach to estimate the diagnostic and therapeutic impact of genomic technology in clinical practice. We would suggest that this is a general approach that would allow new genomic technology to be assessed in pathology. Furthermore, it would be reasonable to review established techniques in the context of these criteria, in particular, it would be reasonable to ask if much of the established immunohistochemistry would show similar clinical impact if tested in the same way.

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**Figure Legends**

**Figure 1. Routine implementation of DNA methylation arrays into a specialist paediatric neuropathology centre.** (A) Schematic representation of the origin of UK cases referred to our practice over a two-year period and included in this study. (B) The proportion of total local cases and referrals for a second opinion. (C) Annual proportion of UK’s cases reported with and without a DNA methylation array (EPIC, 850K) at our neuropathology centre, total numbers shown based on CRUK data[20]. (D) Proportions of arrays performed in all cases reported during two years with a final diagnosis of medulloblastoma, ependymoma, low- and high-grade gliomas.

**Supplementary Figure S1. Indication for requesting a DNA methylation array.** (A) Breakdown of arrays performed by broad diagnosis. (B) Breakdown of arrays performed by indication.

**Figure 2. Diagnostic yield of DNA methylation arrays is highest in well characterised CNS tumours.** (A) Cases broken down by percentage of probes that failed to hybridise, (B) indication for array and (C) by broad diagnosis. Red dotted lines represent the 0.9 calibrated threshold, values equal and above this value were accepted for diagnostic reporting.

**Supplementary Figure S2.** **Other factors considered in relation to diagnostic yield of DNA methylation arrays.** (A) No significant difference was observed for calibrated scores between local and referred cases. (B) No difference was observed for rates between difference age groups.

**Figure 3. DNA methylation classification has an additive diagnostic value in 35% of CNS paediatric cases (n=107).** Graphical representation of overall impact of arrays on routine diagnostic practice for paediatric CNS tumours. Diagnostic refinement in terms of additive molecular data not otherwise available was the biggest impact (99 cases), followed by amended diagnoses for 5 patients. In practice, all arrays are additive as we can infer a CN plot.

**Table 1. Breakdown of cases by impact on diagnosis.** (A) Outputs leading to an amended diagnosis. (B) Outputs considered potentially misleading. (C) Outputs that were of uncertain significance.

**Figure 4. Inferring copy number plots and amplification data from DNA methylation array data.** (A) Example CN plot of a non-WNT/non-SHH Medulloblastoma, molecularly defined as Group 4 by methylation array and with multiple chromosome gains and losses visible and *MYCN* amplification. (B) CN plot for chromosome 2 only with visible *MYCN* amplification, (C) false negative *MYCN* status inferred by DNA methylation array, the corresponding cytogenetic data identified focal MYCN amplification. (D) Representative FISH image for *MYCN* testing using MYCN(2p24) in red and AFF3(2q11) in green. (E) Sensitivity and specificity calculations for *MYCN* and (F) *MYC* amplification inferred by CN plots and compared to reported cytogenetic data, for 62 Medulloblastoma patients.

**Figure 5. DNA methylation profiling is estimated to have impacted therapy for 11 patients.** Graphical representation of the clinical impact of array implementation, considering cases where the data offered additive diagnostic information (n=107).

**Figure 6. Impact of suboptimal scoring predictions on the final reported diagnosis.** (A) One in every 3 suboptimal scoring predictions (range 0 to <0.9) will be misleading. (B) Proportion of misleading predictions by calibrated score range. (C) Median calibrated score by diagnostic impact.

**Figure 7. Modern pathology in combination with DNA methylation profiling can assign a final diagnosis to 74.9% of difficult to diagnose cases.** (A) Review of 203 archival cases using current neuropathology practices and how the diagnosis was reached. Insert represents 61 undiagnosable cases which were further analysed. (B) Breakdown of cases diagnosed by local standard pathology methods without requiring array data (n=100) and (C) cases reviewed that required pathology and array data for a molecular diagnosis (n=35). (D) T-SNE plot of 45 remaining unsolvable cases passing QC which failed to classify (black) overlaid on the DKFZ reference cohort of known CNS tumour subgroups (coloured points). Samples that clustered within or adjacent to known groups were considered to match to the corresponding molecular subgroup.

**References**

[1] S.C. Mack, P.A. Northcott, Genomic Analysis of Childhood Brain Tumors: Methods for Genome-Wide Discovery and Precision Medicine Become Mainstream, Journal of clinical oncology : official journal of the American Society of Clinical Oncology 35(21) (2017) 2346-2354.

[2] G. Bertier, J. Carrot-Zhang, V. Ragoussis, Y. Joly, Integrating precision cancer medicine into healthcare-policy, practice, and research challenges, Genome medicine 8(1) (2016) 108.

[3] S.J. Forrest, B. Geoerger, K.A. Janeway, Precision medicine in pediatric oncology, Current opinion in pediatrics 30(1) (2018) 17-24.

[4] F.M. Ippen, H. Colman, M.J. van den Bent, P.K. Brastianos, Precision Medicine for Primary Central Nervous System Tumors: Are We There Yet?, American Society of Clinical Oncology educational book. American Society of Clinical Oncology. Annual Meeting 38 (2018) 158-167.

[5] D.N. Louis, A. Perry, G. Reifenberger, A. von Deimling, D. Figarella-Branger, W.K. Cavenee, H. Ohgaki, O.D. Wiestler, P. Kleihues, D.W. Ellison, The 2016 World Health Organization Classification of Tumors of the Central Nervous System: a summary, Acta neuropathologica 131(6) (2016) 803-20.

[6] E.C. Schwalbe, J.C. Lindsey, S. Nakjang, S. Crosier, A.J. Smith, D. Hicks, G. Rafiee, R.M. Hill, A. Iliasova, T. Stone, B. Pizer, A. Michalski, A. Joshi, S.B. Wharton, T.S. Jacques, S. Bailey, D. Williamson, S.C. Clifford, Novel molecular subgroups for clinical classification and outcome prediction in childhood medulloblastoma: a cohort study, The Lancet. Oncology 18(7) (2017) 958-971.

[7] A. Korshunov, B. Casalini, L. Chavez, T. Hielscher, M. Sill, M. Ryzhova, T. Sharma, D. Schrimpf, D. Stichel, D. Capper, D.E. Reuss, D. Sturm, O. Absalyamova, A. Golanov, S. Lambo, M. Bewerunge-Hudler, P. Lichter, C. Herold-Mende, W. Wick, S.M. Pfister, M. Kool, D.T.W. Jones, A. von Deimling, F. Sahm, Integrated molecular characterization of IDH-mutant glioblastomas, Neuropathology and applied neurobiology (2018).

[8] D. Sturm, B.A. Orr, U.H. Toprak, V. Hovestadt, D.T.W. Jones, D. Capper, M. Sill, I. Buchhalter, P.A. Northcott, I. Leis, M. Ryzhova, C. Koelsche, E. Pfaff, S.J. Allen, G. Balasubramanian, B.C. Worst, K.W. Pajtler, S. Brabetz, P.D. Johann, F. Sahm, J. Reimand, A. Mackay, D.M. Carvalho, M. Remke, J.J. Phillips, A. Perry, C. Cowdrey, R. Drissi, M. Fouladi, F. Giangaspero, M. Lastowska, W. Grajkowska, W. Scheurlen, T. Pietsch, C. Hagel, J. Gojo, D. Lotsch, W. Berger, I. Slavc, C. Haberler, A. Jouvet, S. Holm, S. Hofer, M. Prinz, C. Keohane, I. Fried, C. Mawrin, D. Scheie, B.C. Mobley, M.J. Schniederjan, M. Santi, A.M. Buccoliero, S. Dahiya, C.M. Kramm, A.O. von Bueren, K. von Hoff, S. Rutkowski, C. Herold-Mende, M.C. Fruhwald, T. Milde, M. Hasselblatt, P. Wesseling, J. Rossler, U. Schuller, M. Ebinger, J. Schittenhelm, S. Frank, R. Grobholz, I. Vajtai, V. Hans, R. Schneppenheim, K. Zitterbart, V.P. Collins, E. Aronica, P. Varlet, S. Puget, C. Dufour, J. Grill, D. Figarella-Branger, M. Wolter, M.U. Schuhmann, T. Shalaby, M. Grotzer, T. van Meter, C.M. Monoranu, J. Felsberg, G. Reifenberger, M. Snuderl, L.A. Forrester, J. Koster, R. Versteeg, R. Volckmann, P. van Sluis, S. Wolf, T. Mikkelsen, A. Gajjar, K. Aldape, A.S. Moore, M.D. Taylor, C. Jones, N. Jabado, M.A. Karajannis, R. Eils, M. Schlesner, P. Lichter, A. von Deimling, S.M. Pfister, D.W. Ellison, A. Korshunov, M. Kool, New Brain Tumor Entities Emerge from Molecular Classification of CNS-PNETs, Cell 164(5) (2016) 1060-1072.

[9] D. Capper, D.T.W. Jones, M. Sill, V. Hovestadt, D. Schrimpf, D. Sturm, C. Koelsche, F. Sahm, L. Chavez, D.E. Reuss, A. Kratz, A.K. Wefers, K. Huang, K.W. Pajtler, L. Schweizer, D. Stichel, A. Olar, N.W. Engel, K. Lindenberg, P.N. Harter, A.K. Braczynski, K.H. Plate, H. Dohmen, B.K. Garvalov, R. Coras, A. Holsken, E. Hewer, M. Bewerunge-Hudler, M. Schick, R. Fischer, R. Beschorner, J. Schittenhelm, O. Staszewski, K. Wani, P. Varlet, M. Pages, P. Temming, D. Lohmann, F. Selt, H. Witt, T. Milde, O. Witt, E. Aronica, F. Giangaspero, E. Rushing, W. Scheurlen, C. Geisenberger, F.J. Rodriguez, A. Becker, M. Preusser, C. Haberler, R. Bjerkvig, J. Cryan, M. Farrell, M. Deckert, J. Hench, S. Frank, J. Serrano, K. Kannan, A. Tsirigos, W. Bruck, S. Hofer, S. Brehmer, M. Seiz-Rosenhagen, D. Hanggi, V. Hans, S. Rozsnoki, J.R. Hansford, P. Kohlhof, B.W. Kristensen, M. Lechner, B. Lopes, C. Mawrin, R. Ketter, A. Kulozik, Z. Khatib, F. Heppner, A. Koch, A. Jouvet, C. Keohane, H. Muhleisen, W. Mueller, U. Pohl, M. Prinz, A. Benner, M. Zapatka, N.G. Gottardo, P.H. Driever, C.M. Kramm, H.L. Muller, S. Rutkowski, K. von Hoff, M.C. Fruhwald, A. Gnekow, G. Fleischhack, S. Tippelt, G. Calaminus, C.M. Monoranu, A. Perry, C. Jones, T.S. Jacques, B. Radlwimmer, M. Gessi, T. Pietsch, J. Schramm, G. Schackert, M. Westphal, G. Reifenberger, P. Wesseling, M. Weller, V.P. Collins, I. Blumcke, M. Bendszus, J. Debus, A. Huang, N. Jabado, P.A. Northcott, W. Paulus, A. Gajjar, G.W. Robinson, M.D. Taylor, Z. Jaunmuktane, M. Ryzhova, M. Platten, A. Unterberg, W. Wick, M.A. Karajannis, M. Mittelbronn, T. Acker, C. Hartmann, K. Aldape, U. Schuller, R. Buslei, P. Lichter, M. Kool, C. Herold-Mende, D.W. Ellison, M. Hasselblatt, M. Snuderl, S. Brandner, A. Korshunov, A. von Deimling, S.M. Pfister, DNA methylation-based classification of central nervous system tumours, Nature 555(7697) (2018) 469-474.

[10] D. Capper, D. Stichel, F. Sahm, D.T.W. Jones, D. Schrimpf, M. Sill, S. Schmid, V. Hovestadt, D.E. Reuss, C. Koelsche, A. Reinhardt, A.K. Wefers, K. Huang, P. Sievers, A. Ebrahimi, A. Scholer, D. Teichmann, A. Koch, D. Hanggi, A. Unterberg, M. Platten, W. Wick, O. Witt, T. Milde, A. Korshunov, S.M. Pfister, A. von Deimling, Practical implementation of DNA methylation and copy-number-based CNS tumor diagnostics: the Heidelberg experience, Acta neuropathologica 136(2) (2018) 181-210.

[11] A.F. Fernandez, Y. Assenov, J.I. Martin-Subero, B. Balint, R. Siebert, H. Taniguchi, H. Yamamoto, M. Hidalgo, A.C. Tan, O. Galm, I. Ferrer, M. Sanchez-Cespedes, A. Villanueva, J. Carmona, J.V. Sanchez-Mut, M. Berdasco, V. Moreno, G. Capella, D. Monk, E. Ballestar, S. Ropero, R. Martinez, M. Sanchez-Carbayo, F. Prosper, X. Agirre, M.F. Fraga, O. Grana, L. Perez-Jurado, J. Mora, S. Puig, J. Prat, L. Badimon, A.A. Puca, S.J. Meltzer, T. Lengauer, J. Bridgewater, C. Bock, M. Esteller, A DNA methylation fingerprint of 1628 human samples, Genome research 22(2) (2012) 407-19.

[12] M.J. Aryee, A.E. Jaffe, H. Corrada-Bravo, C. Ladd-Acosta, A.P. Feinberg, K.D. Hansen, R.A. Irizarry, Minfi: a flexible and comprehensive Bioconductor package for the analysis of Infinium DNA methylation microarrays, Bioinformatics (Oxford, England) 30(10) (2014) 1363-9.

[13] T.R. Hastie T, Narasimhan B, Chu G, Impute: impute: Imputation for microarray data. R package version 1.56.0., (2018).

[14] O.H. Louis DN, Wiestler OD, Cavenee WK, Ellison DW, Figarella-Branger D, Perry A, Reifenberger G, Von Deimling A, WHO Classification of Tumours of the Central Nervous System IARC Press, Lyon 2016.

[15] T.R.C.o. Pathologists, Dataset for tumours of the central nervous system, including the pituitary gland (4th edition), Standards and datasets for reporting cancers, The Royal College of Pathologists, London, 2016, pp. 1-41.

[16] Z.M. Hovestadt V, Conumee: Enhanced copy-number variation analysis using Illumina DNA methylation arrays. R package version 1.10.1.

[17] T.J. Stone, A. Keeley, A. Virasami, W. Harkness, M. Tisdall, E. Izquierdo Delgado, A. Gutteridge, T. Brooks, M. Kristiansen, J. Chalker, L. Wilkhu, W. Mifsud, J. Apps, M. Thom, M. Hubank, T. Forshew, J.H. Cross, D. Hargrave, J. Ham, T.S. Jacques, Comprehensive molecular characterisation of epilepsy-associated glioneuronal tumours, Acta neuropathologica 135(1) (2018) 115-129.

[18] D. Stichel, A. Ebrahimi, D. Reuss, D. Schrimpf, T. Ono, M. Shirahata, G. Reifenberger, M. Weller, D. Hanggi, W. Wick, C. Herold-Mende, M. Westphal, S. Brandner, S.M. Pfister, D. Capper, F. Sahm, A. von Deimling, Distribution of EGFR amplification, combined chromosome 7 gain and chromosome 10 loss, and TERT promoter mutation in brain tumors and their potential for the reclassification of IDHwt astrocytoma to glioblastoma, Acta neuropathologica 136(5) (2018) 793-803.

[19] J.C. Pickles, C. Hawkins, T. Pietsch, T.S. Jacques, CNS embryonal tumours: WHO 2016 and beyond, Neuropathology and applied neurobiology 44(2) (2018) 151-162.

[20] Children's cancers incidence statistics, 2019. <https://www.cancerresearchuk.org/health-professional/cancer-statistics/childrens-cancers/incidence>. (Accessed 01/01/2019 2019).

[21] Overview of the UK population: November 2018, 2018. <https://www.ons.gov.uk/peoplepopulationandcommunity/populationandmigration/populationestimates/articles/overviewoftheukpopulation/november2018>. (Accessed 01/01/2019 2019).

[22] Z. Jaunmuktane, D. Capper, D.T.W. Jones, D. Schrimpf, M. Sill, M. Dutt, N. Suraweera, S.M. Pfister, A. von Deimling, S. Brandner, Methylation array profiling of adult brain tumours: diagnostic outcomes in a large, single centre, Acta neuropathologica communications 7(1) (2019) 24.

[23] D. Sturm, B.A. Orr, U.H. Toprak, V. Hovestadt, D.T. Jones, D. Capper, M. Sill, I. Buchhalter, P.A. Northcott, I. Leis, M. Ryzhova, C. Koelsche, E. Pfaff, S.J. Allen, G. Balasubramanian, B.C. Worst, K.W. Pajtler, S. Brabetz, P.D. Johann, F. Sahm, J. Reimand, A. Mackay, D.M. Carvalho, M. Remke, J.J. Phillips, A. Perry, C. Cowdrey, R. Drissi, M. Fouladi, F. Giangaspero, M. Lastowska, W. Grajkowska, W. Scheurlen, T. Pietsch, C. Hagel, J. Gojo, D. Lotsch, W. Berger, I. Slavc, C. Haberler, A. Jouvet, S. Holm, S. Hofer, M. Prinz, C. Keohane, I. Fried, C. Mawrin, D. Scheie, B.C. Mobley, M.J. Schniederjan, M. Santi, A.M. Buccoliero, S. Dahiya, C.M. Kramm, A.O. von Bueren, K. von Hoff, S. Rutkowski, C. Herold-Mende, M.C. Fruhwald, T. Milde, M. Hasselblatt, P. Wesseling, J. Rossler, U. Schuller, M. Ebinger, J. Schittenhelm, S. Frank, R. Grobholz, I. Vajtai, V. Hans, R. Schneppenheim, K. Zitterbart, V.P. Collins, E. Aronica, P. Varlet, S. Puget, C. Dufour, J. Grill, D. Figarella-Branger, M. Wolter, M.U. Schuhmann, T. Shalaby, M. Grotzer, T. van Meter, C.M. Monoranu, J. Felsberg, G. Reifenberger, M. Snuderl, L.A. Forrester, J. Koster, R. Versteeg, R. Volckmann, P. van Sluis, S. Wolf, T. Mikkelsen, A. Gajjar, K. Aldape, A.S. Moore, M.D. Taylor, C. Jones, N. Jabado, M.A. Karajannis, R. Eils, M. Schlesner, P. Lichter, A. von Deimling, S.M. Pfister, D.W. Ellison, A. Korshunov, M. Kool, New Brain Tumor Entities Emerge from Molecular Classification of CNS-PNETs, Cell 164(5) (2016) 1060-72.

[24] S. Pfister, M. Remke, A. Benner, F. Mendrzyk, G. Toedt, J. Felsberg, A. Wittmann, F. Devens, N.U. Gerber, S. Joos, A. Kulozik, G. Reifenberger, S. Rutkowski, O.D. Wiestler, B. Radlwimmer, W. Scheurlen, P. Lichter, A. Korshunov, Outcome prediction in pediatric medulloblastoma based on DNA copy-number aberrations of chromosomes 6q and 17q and the MYC and MYCN loci, Journal of clinical oncology : official journal of the American Society of Clinical Oncology 27(10) (2009) 1627-36.

[25] A. Mansouri, L.D. Hachem, S. Mansouri, F. Nassiri, N.J. Laperriere, D. Xia, N.I. Lindeman, P.Y. Wen, A. Chakravarti, M.P. Mehta, M.E. Hegi, R. Stupp, K.D. Aldape, G. Zadeh, MGMT promoter methylation status testing to guide therapy for glioblastoma: refining the approach based on emerging evidence and current challenges, Neuro-oncology (2018).

[26] A.M. Buccoliero, F. Castiglione, D. Rossi Degl'Innocenti, M. Paglierani, V. Maio, C.F. Gheri, F. Garbini, D. Moncini, A. Taddei, I. Sardi, M. Sanzo, F. Giordano, F. Mussa, L. Genitori, G.L. Taddei, O6-Methylguanine-DNA-methyltransferase in recurring anaplastic ependymomas: PCR and immunohistochemistry, Journal of chemotherapy (Florence, Italy) 20(2) (2008) 263-8.

[27] D.T. Nagasawa, A. Trang, W. Choy, M. Spasic, A. Yew, G. Zarinkhou, H.M. Garcia, I. Yang, Genetic expression profiles of adult and pediatric ependymomas: molecular pathways, prognostic indicators, and therapeutic targets, Clinical neurology and neurosurgery 115(4) (2013) 388-99.

[28] A. Korshunov, M. Ryzhova, V. Hovestadt, S. Bender, D. Sturm, D. Capper, J. Meyer, D. Schrimpf, M. Kool, P.A. Northcott, O. Zheludkova, T. Milde, O. Witt, A.E. Kulozik, G. Reifenberger, N. Jabado, A. Perry, P. Lichter, A. von Deimling, S.M. Pfister, D.T. Jones, Integrated analysis of pediatric glioblastoma reveals a subset of biologically favorable tumors with associated molecular prognostic markers, Acta neuropathologica 129(5) (2015) 669-78.

[29] D.D. Eisenstat, I.F. Pollack, A. Demers, M.V. Sapp, P. Lambert, J.D. Weisfeld-Adams, P.C. Burger, F. Gilles, R.L. Davis, R. Packer, J.M. Boyett, J.L. Finlay, Impact of tumor location and pathological discordance on survival of children with midline high-grade gliomas treated on Children's Cancer Group high-grade glioma study CCG-945, Journal of neuro-oncology 121(3) (2015) 573-81.

[30] R. Banan, A. Christians, S. Bartels, U. Lehmann, C. Hartmann, Absence of MGMT promoter methylation in diffuse midline glioma, H3 K27M-mutant, Acta neuropathologica communications 5(1) (2017) 98.

[31] E. Uro-Coste, J. Masliah-Planchon, A. Siegfried, M. Blanluet, S. Lambo, M. Kool, T. Roujeau, S. Boetto, G. Palenzuela, A.I. Bertozzi, M. Gambart, I. Coupier, I. Oliver-Petit, L. Golmard, S. Julia, F. Savagner, B. Mohand-Oumoussa, A. Tauziede-Espariat, M.B. Delisle, D. Figarella-Branger, F. Bourdeaut, V. Rigau, ETMR-like infantile cerebellar embryonal tumors in the extended morphologic spectrum of DICER1-related tumors, Acta neuropathologica 137(1) (2019) 175-177.