The potential for epigenetic analysis of paediatric CNS tumours to improve diagnosis, treatment and prognosis

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Tumours of central nervous system (CNS) origin are the second most prevalent group of cancers in children, yet account for the majority of childhood cancer-related deaths. Such tumours show diverse location, cell type of origin, disease course and long-term outcome, both across and within tumour types, making treatment problematic and contributing to the relatively modest progress in reducing mortality over recent decades. As technological advances begin to reveal the genetic landscape of all cancers, it is becoming increasingly clear that genetic disruption represents only one ‘layer’ of molecular disruption associated with disease aetiology. Obtaining a full understanding of tumour behaviour requires an understanding of the cellular and molecular pathways disrupted during tumourigenesis, particularly in relation to gene expression. The utility of such an approach has allowed stratification of cancers such as medulloblastoma into subgroups based on molecular features, with potential to refine risk prediction. Given that epigenetic disruption is a universal feature of all human cancers, it is logical to speculate that interrogating epigenetic marks may help to further define the molecular profile, and therefore the clinical trajectory, of tumours. An integrated approach to build a molecular ‘signature’ of individual tumours that incorporates traditional morphological and demographic information, genetic and transcriptome analysis, in addition to epigenomics (DNA methylation and non-coding RNA analysis), offers tremendous promise to (i) inform treatment approach, (ii) facilitate accurate early identification (preferably at diagnosis) of variable risk groups (both good and poor prognosis groups), and (iii) track disease progression in childhood CNS tumours.

Key words: paediatric, CNS tumour, DNA methylation, epigenetics

introduction

Tumours of the central nervous system (CNS) account for 20%–25% of all paediatric cancer diagnoses in the developed world and occur at an incidence of 5 children per 100 000 [1–3]. CNS tumours are the second most prevalent group of cancers in children, after leukaemias, but account for more cancer-related deaths [2–4]. This represents approximately one-third of cancer-associated deaths [4–6]. In addition to their relatively high mortality, CNS tumours are associated with extensive morbidities such that 90% of survivors are left with long-term neurocognitive and psycho-social deficits. These include, but are not limited to, pituitary dysfunction, growth hormone deficiency, epilepsy, vision loss, impaired motor skills, memory dysfunction, attention and behavioural disorders and reduced IQ [7–9]. Given the devastating outcomes associated with this disease, reducing mortality and improving long-term outcomes for survivors is critical. To achieve this, we must advance our current understanding of the molecular pathways associated with disease aetiology, which in turn will facilitate the identification of reliable biomarkers to (i) inform treatment approach, (ii) identify early (preferably at diagnosis) cases with a good or poor prognosis and (iii) allow for disease monitoring.

classification of CNS tumours

Diagnosis of paediatric CNS tumours is traditionally based upon cell morphology and location, with tumours broadly classified according to World Health Organisation (WHO) criteria. These classes are the neuroepithelial, cranial and paraspinal nerve, meningeal, lymphoma and haematopoietic, germ cell, sellar region and metastatic. Each is further sub-classified (detailed in Table 1) and graded from stage I to IV to indicate degree of malignancy and aggressiveness [10]. Paediatric CNS tumours show diverse cell type of origin, location, disease course and long-term outcome, with enormous variability in these parameters across and within tumour types. For example medulloblastoma of the posterior fossa may appear as classic,
anaplastic or nodular desmoplastic microscopically, yet prognosis can be independent of these morphological features [11]. The current tumour classification system largely determines treatment approach. This is problematic as such features are not always indicative of tumour behaviour and disease course. Clinical parameters, such as age at diagnosis and presence of metastases, can further inform treatment approach, but are not sufficient to accurately predict outcome. At present, there is no highly accurate, unbiased test to reliably predict outcome and a more sensitive and specific approach is urgently required.

the genetic landscape of paediatric CNS tumours

As with many adult tumours, the variable behaviour of paediatric CNS tumours may be due (at least in part) to distinct underlying genetic deficits. Traditional studies have relied on cytogenetic and copy number analysis to identify gross genetic aberrations, such as gain of chromosome 1q, loss of 16q and amplification of PDGFRA in paediatric high-grade glioma (HGG) [12, 13]. A growing number of large collaborative projects have begun profiling the genetic landscape of both adult and paediatric CNS tumours in order to more fully explore their features. The emerging picture emphasizes some key differences. For example whereas adult HGG is characterised by mutations in IDH1 and amplification of EGFR, such alterations are not a common feature in the childhood equivalent [12]. Instead, childhood HGG display mutations in chromatin modifiers and epigenetic regulation, such as H3F3A, ATRX and DAXX [14]. Such differences are not altogether surprising given the differing incidence and prognosis of tumours in adults relative to children. For example low-grade gliomas (LGGs) are more common in children than adults and the reverse is true for HGGs such as glioblastoma (GBM) [15, 16]. Additionally, HGG arise almost exclusively as de novo lesions in children in contrast to the adult setting where ~50% arise from a previous low-grade astrocytoma
Established clinical and genetic differences between paediatric and adult CNS tumours are shown in Table 2. As well as their utility in diagnosis, genetic alterations have utility in prognostication. In ependymoma for example, gain of chromosome 1q is significantly associated with poor outcome [23–26]. Additionally, paediatric HGGs can be divided into three distinct groups based on copy number changes and these groups have distinct prognoses, with gain of 7q and loss of 10q having a poor prognosis [12, 27]. Despite the array of cytogenetic and genetic alterations detected in paediatric CNS tumours (Table 3), up to 70% of tumours display balanced genomic profiles [12, 28, 37], suggesting a role for additional sub-microscopic point mutations, or alternative non-genetic (epigenetic) mechanisms in tumour aetiology.

the potential for epigenetic profiling to refine molecular classification to better inform treatment approach and predict outcome

Despite progress in understanding the genetic landscape of childhood CNS tumours, there remains a large proportion of cases with few if any genetic alterations. Additionally, few specific genetic variant(s) and gene expression signatures have yet been identified which can be used in diagnosis or to predict long-term outcome with a high degree of accuracy. As such, recent studies have begun to explore the clinical utility of epigenetic variation in CNS tumours.

Epigenetics is the study of modifications to DNA which alter the activity state of the underlying DNA in the absence of DNA sequence change [39]. Epigenetic modifications include DNA methylation, histone modification and chromatin remodelling, all of which have the capacity to alter gene expression and regulate genome stability [39]. Ongoing research has revealed a range of epigenetically silenced and activated genes in cancer and observed the effects of these changes on various signalling processes [40, 41].

DNA methylation has been extensively studied in adult cancer and it is now well established that virtually all human tumours exhibit aberrant DNA methylation patterns [42]. Neoplastic cells typically exhibit a pattern of genome-wide hypomethylation and promoter-specific hypermethylation [43]. Whereas the former is thought to contribute to tumourigenesis by causing chromosomal instability, translocations, gene disruption and reactivation of endoparasitic sequences (such as LINE and SINE elements) [43–45], the latter is associated with silencing of a wide range of genes involved in tumour suppression.

Studies of DNA methylation in tumour development and progression have contributed greatly to our understanding of the neoplastic process [42]. However, DNA methylation also has the potential to identify novel diagnostic and prognostic biomarkers. Genes that are differentially methylated between malignant and healthy tissue (such as GSTPI, hypermethylated in 80%–90% of malignant neoplastic but not in benign hyperplastic prostate tissue) have the potential to be used as diagnostic biomarkers of cancer [46]. Additionally, the methylation status of specific genes may predict outcome. For example hypermethylation of DAPK and CDKN2A are linked to poorer outcome in lung and colorectal cancer, whereas hypermethylation of MGMT predicts a favourable outcome in adult HGG [46–56]. Such studies emphasize the potential of DNA methylation analysis to enhance our understanding of cancer development and biology and improve diagnostic and prognostic methods. Despite this, there are very few DNA methylation biomarkers currently being used routinely in clinical practice; therefore, further investigation is required. Further, DNA methylation has not yet been extensively

The current classification system of medulloblastoma defines four subgroups according to gene expression profile, although it is likely that in future each subgroup will be further stratified into additional subtypes. These are WNT, SHH, Group 3 and Group 4, defined by combining the results of a number of large expression profiling studies. WNT and SHH subgroups display up-regulation of the WNT and SHH signalling pathways, respectively, Group 3 show up-regulated photoreceptor/GABAergic signalling, and Group 4 display up-regulated neuronal/glutamatergic signalling. Each subgroup also has distinct cytogenetic and genomic alterations, (Table 3) and distinct ages of onset and disease prognosis. WNT tumours typically occur in children and have a very good prognosis. Group 3 and 4 also primarily occur in children but confer a poor and intermediate prognosis, respectively. The SHH subgroup typically affects infants and adults and confers a good prognosis in infants and an intermediate prognosis in adults [11]. Such subgroups are not definitive however, as there is some difficulty assigning tumours to Group 3 or 4. Despite this, and similarly to the findings in ependymoma, the identification of such subgroups has the potential to improve diagnosis, treatment and prognosis.
Table 2. Comparison of paediatric and adult CNS tumour features

<table>
<thead>
<tr>
<th>Tumour type</th>
<th>Occurrence (% of all paediatric/adult CNS tumour diagnoses)</th>
<th>Prognosis (5-year overall survival rate)</th>
<th>Genetics and development</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Paediatric: 20%</td>
<td>Paediatric: 96.8%</td>
<td>Paediatric: typically 0–1 chromosome change involving +5 and +7</td>
</tr>
<tr>
<td></td>
<td>Adult: 0.5%</td>
<td>Adult: 84.4%</td>
<td>Adult: typically 4–9 chromosome changes involving +5, +6 and +7 [17]</td>
</tr>
<tr>
<td></td>
<td>Paediatric: 2%</td>
<td>Paediatric: 31%</td>
<td>Paediatric: commonly exhibit stable genome, +1q, −16q, H3F3A mutation</td>
</tr>
<tr>
<td></td>
<td>Adult: 2%</td>
<td>Adult: 18.9%</td>
<td>Adult: rarely exhibit stable genome, +7, −10q, EGFR amplification, IDHI mutation [13]</td>
</tr>
<tr>
<td>Glioblastoma</td>
<td>Paediatric: 3.5%</td>
<td>Paediatric: 19.2%</td>
<td>Paediatric: arise de novo (primary)</td>
</tr>
<tr>
<td></td>
<td>Adult: 18%</td>
<td>Adult: 5.8%</td>
<td>Adult: 50% develop from previous low-grade astrocytoma (secondary) [13]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>As per anaplastic astrocytoma</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Adult: IDH1 mutations are more prevalent in secondary than primary glioblastoma</td>
</tr>
<tr>
<td>Oligodendroglial tumours</td>
<td>Paediatric: 1.6%</td>
<td>Oligodendroglioma</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Adult: 2.0%</td>
<td></td>
<td>Paediatric: polysomy 1p and 19q</td>
</tr>
<tr>
<td></td>
<td>Paediatric: 92.2%</td>
<td></td>
<td>Adult: −1p, −19q, TF53 mutation [18]</td>
</tr>
<tr>
<td></td>
<td>Adult: 62.4%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Anaplastic oligodendroglioma</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Paediatric: no data</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Adult: 43.9%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oligoastrocytic tumours</td>
<td>Paediatric: 0.9%</td>
<td>Paediatric: 81.2%</td>
<td>no information available</td>
</tr>
<tr>
<td></td>
<td>Adult: 1.0%</td>
<td>Adult: 47%</td>
<td></td>
</tr>
<tr>
<td>Ependymal tumours</td>
<td>Ependymoma and Anaplastic ependymoma</td>
<td>Paediatric: 74.7%</td>
<td>Paediatric: no chromosomal changes in 36–58% of patients, +1q, −1p, −3, −6, −9p, −17</td>
</tr>
<tr>
<td></td>
<td>Paediatric: 6.7%, commonly located in the posterior fossa</td>
<td>Adult: 47%</td>
<td>Adult: chromosomal changes in &gt;90% of patients, +2, +5, +12, +18, +X, −10, −13q, −14q, NF2 mutation [19]</td>
</tr>
<tr>
<td></td>
<td>Adult: 1.9%, commonly located in supratentorial regions and the spinal cord</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Choroid plexus tumours</td>
<td>All choroid plexus tumours</td>
<td>no information available</td>
<td>Choroid plexus papilloma</td>
</tr>
<tr>
<td></td>
<td>Paediatric: 2.4%</td>
<td></td>
<td>Paediatric: +12</td>
</tr>
<tr>
<td>Embryonal tumours</td>
<td>Adult: 0.1%</td>
<td></td>
<td>Adult: +5q, +6q [20]</td>
</tr>
<tr>
<td></td>
<td>Paediatric: 15.8%</td>
<td>Paediatric: 61.9%</td>
<td>Medulloblastoma</td>
</tr>
<tr>
<td></td>
<td>Adult: 0.4%</td>
<td>Adult: 58.1%</td>
<td>Paediatric: MYCN amplification, −10q</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Adult: CDK6 amplification [21, 22]</td>
</tr>
</tbody>
</table>

All data for occurrence and prognostic rates adapted from the Central Brain Tumor Registry of the United States statistical report 2006–2010 [16].
### Table 3. Known genetic variants in paediatric CNS tumours

<table>
<thead>
<tr>
<th>Tumour type</th>
<th>Genomic alterations$^a$</th>
<th>Chromosomal changes</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Astrocytic tumours</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pilocytic astrocytoma</td>
<td>$NF1, BRAF, RAFI, KRAS, PTPN11$</td>
<td>$+1, +3, +4, +6, +9, +10, +11, +12, +13, +14, +15, +16, +17,$</td>
<td>[28]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$+19, +20, +21, +22, +X, −7, −8, −17$</td>
<td></td>
</tr>
<tr>
<td>Pleomorphic xanthoastrocytoma$^b$</td>
<td></td>
<td>$−9, −17, +X$</td>
<td>[29]</td>
</tr>
<tr>
<td>Anaplastic astrocytoma</td>
<td>$TP53, H3F3A, PIK3CA, PDGFRA, CDKN2A/2B$</td>
<td>$+1q, −16q$</td>
<td>[12, 13, 27, 30]</td>
</tr>
<tr>
<td>Glioblastoma</td>
<td>$TP53, H3F3A, PIK3CA, PDGFRA, CDKN2A/B$</td>
<td>$+1q, −16q$</td>
<td>[12, 13, 27, 30]</td>
</tr>
<tr>
<td>Ependymal tumours</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ependymoma and anaplastic ependymoma</td>
<td>$Notch1$</td>
<td>$+1q, +7, +9, −1p, −3, −6, −9p, −13q, −17, −22$</td>
<td>[19, 26, 31]</td>
</tr>
<tr>
<td>Choroid plexus tumours</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Choroid plexus papilloma</td>
<td></td>
<td></td>
<td>[20]</td>
</tr>
<tr>
<td>Choroid plexus carcinoma$^b$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Embryonal tumours</td>
<td></td>
<td></td>
<td>[11]</td>
</tr>
<tr>
<td>Medulloblastoma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WNT subgroup</td>
<td>$CTNNB1, DDX3X, SMARCA4, MLL2, TP53$</td>
<td>$−6$</td>
<td></td>
</tr>
<tr>
<td>SHH subgroup</td>
<td>$PTCH1, MLL2, TP53, DDX3X, BCOR, LDB1, TCF4, MYCN, GLI2$</td>
<td>$+3q, +9p, −9q, −10q, −14q, −17p$</td>
<td></td>
</tr>
<tr>
<td>Group 3 subgroup</td>
<td>$SMARCA4, MLL2, SPTB, CTDNEP1, LRP1B, TNXB, MYC, PVT1, OTX2$</td>
<td>$+1q, +7q, +17q, +18, −8, −10q, −11, −16q, −17p$</td>
<td></td>
</tr>
<tr>
<td>Group 4 subgroup</td>
<td>$KDM6A, MLL3, ZMYM3, CBFA2T2, SNCAIP, MYCN, CDK6$</td>
<td>$+4, +7, +17q, +18, −8, −10, −11, −17p$</td>
<td></td>
</tr>
<tr>
<td>CNS primitive neuroectodermal tumour</td>
<td>$MYC, MYCN, C19MC, CDKN2A/2B$</td>
<td>$+1q, +2p, −13q, −16p, −19p, polysomy 2, polysomy 8$</td>
<td>[32–34]</td>
</tr>
<tr>
<td>Atypical teratoid/rhabdoid tumour</td>
<td>$INI1, SMARCB1$</td>
<td>$−22q$</td>
<td>[35, 36]</td>
</tr>
</tbody>
</table>

$^a$Genomic alterations include single-nucleotide variants, indels, focal amplification or gain and focal homozygous or hemizygous deletion.

$^b$Mixed adult/paediatric cohort.
investigated in paediatric CNS tumours, despite emerging data suggesting a key role in tumour development and progression with utility for refining diagnostic and prognostic testing.

medulloblastoma

The majority of DNA methylation studies of paediatric medulloblastoma have been loci specific, concentrating primarily on well-known tumour suppressor genes identified in adult studies, such as RASSF1A, p16\textsuperscript{INK4A}, p14\textsuperscript{ARF}, CDH1 and HIC1. Hypermethylation of the promoter region of RASSF1A, seen in many adult cancers [57], has been reported in 88% (14/16), 100% (25/25), 90% (19/21) and 93% (41/44) of medulloblastoma cases examined [58–61]. In contrast to RASSF1A, other tumour suppressor methylation studies have yielded conflicting results. Hypermethylation of p14\textsuperscript{ARF}, commonly identified as an early-stage event in a variety of adult cancers [62–67], has been variously reported in 75% (3/4), 63% (7/11) and 40% (17/43) of medulloblastomas [68–70]. In contrast, four additional studies found little or no evidence of p16\textsuperscript{INK4A} hypermethylation [58, 61, 71, 72]. Conflicting results have also been reported in medulloblastoma for p14\textsuperscript{ARF} [61, 69–71]. HIC1, known to be hypermethylated in a variety of cancers including colon, breast, adult brain and leukaemia [73–75], has been shown to be hypermethylated in paediatric medulloblastoma in 37% (17/44) and 85% (33/39) of cases [61, 76]. The latter study also identified a significant correlation between hypermethylation and reduced gene expression. Finally, CDH1 has been investigated in three paediatric medulloblastoma studies, reporting promoter hypermethylation in 0% (0/16), 9% (4/46) and 76% (31/41) of tumours [58, 70, 72]. As the understanding of the genetics of paediatric medulloblastoma has increased, studies have begun to explore the extent of DNA methylation of key driver genes in particular medulloblastoma subgroups. For example Pritchard and Olson [77] examined DNA methylation in the PTCH1–1B gene in four tumour samples, with reduced expression of the PTCH1 gene, yet no evidence of promoter hypermethylation in PTCH1–1B was found [77]. In contrast, Diede et al. [78] identified PTCH1–1C promoter hypermethylation and decreased expression of PTCH1 in three of four medulloblastoma samples tested [78].

The advent of robust technologies for measuring DNA methylation at the genome-scale promises to facilitate rapid translation of epigenetic data into clinical utility. For example a relatively low-resolution analysis of 1505 CpG sites in 230 medulloblastomas allowed assignment of 216 tumours to one of the four established subgroups based solely on methylation status [79]. DNA methylation analysis of over 450 000 CpG sites in 276 medulloblastomas identified a 48-CpG site signature that classified all tumours into one of the four established subgroups (97.6% accuracy) [80]. In addition to classifying tumours, identification of novel epigenetic signatures associated with specific subtypes reveals potential novel cellular pathways associated with disease progression. This is particularly relevant for subgroups 3 and 4 which are less well characterised and have fewer common gene mutations than the SHH and WNT groups.

CNS primitive neuroectodermal tumour

DNA methylation studies in primitive neuroectodermal tumour (CNS PNET) have focussed on tumour suppressor genes previously identified as aberrantly methylated in cancer. These include RASSF1A, p14\textsuperscript{ARF} and p16\textsuperscript{INK4A}. Fairly consistent hypermethylation of the RASSF1A promoter in 79% (19/24), 83% (5/6) and 67% (6/9) of cases has been identified [59, 60, 81]. In contrast, a consistent lack of hypermethylation of the p16\textsuperscript{INK4A} promoter has been reported [70, 71]. Finally, p14\textsuperscript{ARF} has been shown to be variably hypermethylated in 50% (3/6) and 0% (0/4) of cases [70, 71]. A wider methylation screen by Schwalbe et al. [82] of 1505 CpG sites between 29 CNS PNET and 136 medulloblastoma,ependymomas and HGGs did not cluster CNS PNETs as one group; instead, they were interspersed between the other tumour types based on methylation profile [82], the significance of which remains unclear.

atypical teratoid/rhabdoid tumour

Just two small studies in DNA methylation have been undertaken in atypical teratoid/rhabdoid tumour (AT/RT). This research demonstrated hypermethylation of the RASSF1A tumour suppressor in 67% (4/6) of cases [81], and hypermethylation of the telomerase enzyme gene \( hTERT \) in 75% (27/36) of cases [83].

pilocytic astrocytoma

Despite its high prevalence, DNA methylation in pilocytic astrocytoma (PA) has not been extensively investigated. Locus-specific methylation identified promoter hypermethylation of \( PTEN \) in 35% (6/17) of cases [84], while promoter hypermethylation of \( hTERT \) has been reported in 1% (67/68) of cases tested [83]. \( hTERT \) expression levels of these samples were then compared with those of a group of predominantly hypermethylated HGGs (39 of 43 cases with >15% methylation) and were found to have significantly lower gene expression [83]. Recently, a genome-wide methylation investigation identified two subgroups of PA according to location (infratentorial and supratentorial) [85]. Differentially methylated genes included \( MASP1, PRKCDBP, GP6 \) and \( ACSBG2 \), with evidence of differential MASP1 expression between location subgroups [85].

high-grade glioma

Most DNA methylation studies in HGG have been adult focused, identifying promoter hypermethylation in many genes including \( CRABP2, SOCS3, SLC22A18, EGFR, SGNE1, RB1, EMP3, RASSF1A, BLU, p16\textsuperscript{INK4A}, p15\textsuperscript{INK4b}, DAPK, TIP3P, CDH1, CD81, PRKCDBP, MEST, TNSRF5A0, F2D9, MGMT, AREG, HOXA11, hMLH1, NDRG2, NPTX2 \) and \( TES \) [86–90]. Concomitant changes in gene expression (\( TES, SOCS3, CRABP2 \) and \( SLC22A18 \)) [90–92] and association with poorer prognosis (\( AREG, SOCS3 \) and \( MGMT \)) [86, 93] have also been noted. Recent genome-wide profiling in adult GBM revealed a subset of tumours displaying a CpG island methylator, or CIMP\textsuperscript{+} phenotype, characterised by a large number of hypermethylated genes [94]. CIMP\textsuperscript{+} tumours also showed specific \(+8q \) and \(+10q \) copy number alterations, increased \( IDH1 \) mutations and better prognosis.

The methylation status of \( MGMT \), encoding an important DNA repair enzyme, has been studied extensively in adult HGG.
More than 15 studies have reported promoter hypermethylation in 36%–68% of samples [47–56, 89, 95–99]. Associated with prolonged survival in many instances [47–56], MGMT promoter hypermethylation likely decreases MGMT protein levels compromising repair of DNA damage, induced by alkylating chemotherapeutics. One of these agents, Temozolomide, is often used in adult HGG, particularly in patients with hypermethylation of MGMT, where it may improve patient survival [50, 53, 54]. Ideally, the correlation between MGMT hypermethylation and improved clinical outcome would translate from the adult to the paediatric setting. However, results to date have not been definitive. A 2010 study of 22 relapsed HGGs of mixed subtype identified MGMT promoter hypermethylation in 77% (17/22) of cases. The study also found a significant positive correlation between promoter hypermethylation and longer survival time [100]. However, two further studies in 2011 showed lower instances of hypermethylation of 0% (0/10) and 16% (3/19) [101, 102]. The latter also examined the association with clinical outcome and found no significant correlation between the two. Thus, the association of MGMT methylation with outcome in HGG remains unclear in a paediatric setting.

As with PA, hTERT and PTEN methylation has also been investigated in HGG, revealing promoter hypermethylation of hTERT in 90% (39/43) of HGGs and promoter hypermethylation of PTEN in 33% (2/6) of grade III and 33% (3/9) of grade IV HGGs [83, 84]. The 39 hTERT hypermethylated samples also expressed hTERT at a significantly higher level than hypomethylated HGG and a group of 67 hypomethylated PAs [83].

**ependymoma**

RASSF1A promoter hypermethylation has been reported in 86% (30/35) of a mixed ependymal cohort of adult and paediatric, multiple location and histopathological types of tumours (spinal and intracranial and classic, anaplastic and myxopapillary). High rates of hypermethylation were found irrespective of age [103], but a lower incidence of hypermethylation, 56% (15/27) of cases, was subsequently reported in paediatric ependymoma. The same study compared methylation status with clinical outcome and found no significant association [104]. p14ARF and p16INK4A appear to be unmethylated in the majority of ependymomas [103–105]; however, a further study identified p14ARF promoter hypermethylation in 21% (23/108) of cases [106]. Finally, HIC1 has been investigated in one study, finding promoter hypermethylation in 85% (23/27) of cases. No significant correlation could be found between hypermethylation and decreased gene expression [107].

As ependymomas, like HGGs, are a type of glial cell tumour, MGMT promoter methylation has also been investigated. Five studies have reported varying incidence of hypermethylation of 27% (39/142), 23% (3/13), 5% (1/20), 4% (1/27) and 0% (0/9) of cases, suggesting MGMT methylation is not a prominent feature of paediatric ependymoma [103–105, 108, 109].

A single genome-wide study identified 63% (37/59) of ependymoma cases as having hTERT promoter hypermethylation. An investigation of 45 posterior fossa ependymomas found a significant difference in OS between hypermethylated and non-hypomethylated cases, with an average 5-year OS rate of 51% in hypermethylated cases, and an average 5-year OS rate of 95% in non-hypomethylated cases. However, after multivariate analysis, hypermethylation was only a marginally independent predictor of survival (P = 0.055) [83].

Recently, genome-wide methylation analysis of both adult and paediatric ependymoma has revealed three distinct subgroups associated with supratentorial, posterior fossa and mixed spinal/posterior fossa location [110]. With gene expression data, methylation data showed posterior fossa tumours to split into two groups, a pure posterior fossa group, group PFA, and a mixed spinal/posterior fossa group, group PFB. PFA tumours were defined as CIMP positive, due to their greater number of hypermethylated regions. In contrast to studies in GBM where the CIMP+ phenotype was associated with improved survival, the CIMP− phenotype in ependymoma may confer a worse prognosis [110].

**the future: integrating epigenetic and other molecular data—a systems biology approach to understanding CNS tumours**

DNA methylation analysis has great potential in identification of biomarkers for disease diagnosis and prognosis, and improving understanding of the biology and development of cancer. However, at present, the clinical utility of DNA methylation profiling for CNS tumours remains unclear. Even for the best characterised tumour, medulloblastoma, methylation analysis to date has yielded conflicting results across studies. While RASSF1A is consistently hypermethylated, other well-known tumour suppressor genes examined seem to be variably methylated. Given that the majority of studies described thus far have not investigated the association between methylation status and specific clinical parameters, and those which have did not find any significant associations, it is difficult to pinpoint the source of inter-study variability. It is likely that varying sample sizes (from 4 to 70 cases) contributed, as well as differences in tumour tissue collection and processing protocols. Additionally, it is possible that the cohorts comprised different fractions of tumour ‘subgroups’ (some as yet undefined) likely to have different epigenetic profiles. With appropriate (as large as possible) sample sizes, there is an unprecedented opportunity to identify the underlying epigenetic processes that may contribute to variable outcome among (i) different tumour types and (ii) tumours similarly classified at the time of diagnosis. As such, there is also the considerable potential to further refine biomarkers for subtype classification and therefore to simplify current subtyping procedures.

As with genetic and/or gene expression variation reported in isolation, to date there have been few methylation aberrations consistently associated with specific CNS tumour types, treatment responsiveness or long-term outcome. This parallels the situation with most adult cancers and suggests that an approach that focuses on one type of molecular disruption (e.g. DNA methylation) in isolation is unlikely to fully explain the aetiology of paediatric tumours. Hence, in order to further our understanding, it is essential to integrate analyses of DNA methylation, gene expression and genetic variation (at a minimum). Sturm et al. [111] present such a combined analysis on 210 adult and paediatric GBMs. The study combined epigenetic, copy number, expression and genetic analyses to profile a combined adult and paediatric GBM cohort and identified six subgroups with distinct DNA
methylation and molecular alterations. Similar to previous studies, paediatric patients tended to display mutations in H3F3A and TP53 and amplification of PDGFRA or alternatively minimal copy number variants, as well as aberrant methylation [111]. Although the study integrated extensive DNA methylation data into their analysis, there was no locus examination, which may be useful in further defining subgroups. The study also identified each subgroup as having distinct clinical features including age at onset, tumour location and disease outcome that may be useful in prognosis [111]. Although limited in scope, this is an excellent example of the utility of combining epigenetic and genetic analyses to further our understanding of paediatric CNS tumours, and improve diagnosis, treatment and prognosis.

The 2007 WHO Classification of Central Nervous System Tumours described medulloblastoma as a single entity, and the next imminent edition will describe clinically and molecularly distinct subgroups. Such advances in our understanding emphasize the rapid progress in molecular analysis made over the last 10 years. As the field continues to progress, it is likely that within a short timeframe patients will be provided with a truly personalised approach to diagnosis and treatment, involving full genome sequencing of tumour and germline tissues, as well as bp resolution of DNA methylation (see Figure 1). The biggest challenge we face in implementing such a system is to develop analytical approaches to integrate the vast amount of data into a cohesive model for diagnosis and prognosis, and then translating that model into the clinic to improve outcome and minimise long-term morbidities for these children.

The key to developing a system of personalised medicine is to conduct and integrate the various analytical approaches of clinical, pathology and laboratory-based research teams. Central to this will be the role of the bioinformatician/biostatistician, whose role in integrating and interpreting such data so as to make it clinically meaningful is fundamental to translation in this domain. However, there are several obstacles to conducting such research that must be overcome, namely, obtaining sufficient sample sizes, using consistent methodology, and covering associated costs. Fortunately, these obstacles can be addressed primarily by willing collaboration between research groups. Given the rarity of paediatric CNS tumours, it is unlikely that individual researchers will be able to compile large enough specimen cohorts to make any meaningful discoveries; therefore, researchers must combine their cohorts to create large, detailed study populations for analysis. Collaboration will also give rise to more streamlined methods for tumour storage and processing. Costs associated with the aforementioned analyses can run into the hundreds of dollars per sample, thousands if including cell line or xenograft generation; however, by combining resources or allocating specific analyses to individual research groups, an enormous amount of data can be generated. The Sturm et al. study described above [111] is a perfect example of such a successful collaboration, where tumours analysed were collected from Germany, Canada and Russia and researchers hailed from all over Europe, America and Canada. While tumour biology, like all medical research, is increasingly competitive in terms of attracting funding, an internationally collaborative, harmonised systems biology approach to

![Figure 1. The future of medulloblastoma diagnosis and treatment.](https://example.com/figure1.png)
characterise childhood CNS tumours remains the most promising approach towards making progress in the disproportionately large numbers of children and families confronted with this devastating diagnosis on a daily basis.

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**references**


