Genome-wide DNA methylation profiling for central nervous system embryonal tumours in children: abridged secondary publication

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KEY MESSAGES

1. Genome-wide DNA methylation profiling enables tumour classification of central nervous system (CNS) tumour tissues.
2. Methylation profiling demonstrates utility in the diagnostic process of CNS embryonal tumours, facilitating tumour subgrouping in two-thirds of patients while allowing consideration of alternative diagnoses in one-tenth of patients.
3. Epigenome-based classification of CNS embryonal tumours stratified patients into prognostically relevant disease subgroups.

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Introduction

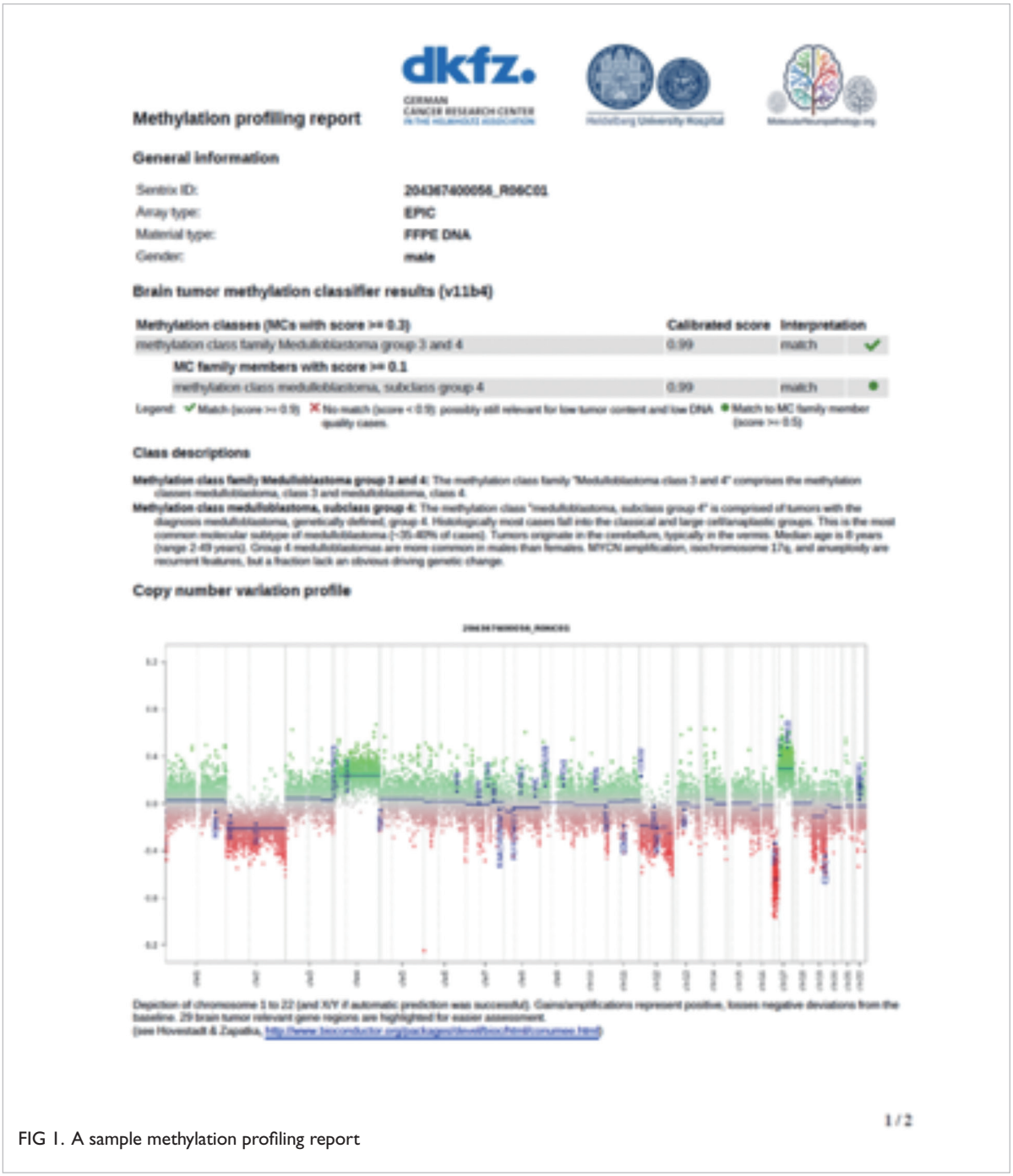
Central nervous system (CNS) tumours are the most common solid tumours in children, with the highest mortality among various paediatric malignancies.[1] In particular, CNS embryonal tumours are associated with poor survival despite the availability of multi-modal treatment with maximal surgical resection, radiation therapy, and chemotherapy. In addition, survivors of such conditions often experience long-term treatment-related toxicity, which negatively impacts their quality of life. The difficulty managing paediatric CNS embryonal tumours is partly related to their molecular heterogeneity. Advances in high-throughput genomic techniques, specifically DNA methylation arrays, have revealed important molecular subgroups within histologically defined entities. CNS embryonal tumours and associated high-grade neuroepithelial tumours (HGNETs), including medulloblastoma, CNS-primitive neuroectodermal tumour (PNET), atypical teratoid/rhabdoid tumour (ATRT), and pineoblastoma, have been classified as clinically relevant epigenomic subgroups.[2] Such molecular classifications have been incorporated into iterations of the World Health Organization (WHO) CNS Tumour Classification.

DNA methylation arrays are increasingly utilised to aid clinical diagnosis, treatment decision, and study trial design. However, their clinical value is dependent on pre-existing diagnostic infrastructure and expertise. We successfully profiled archival tumour tissue from a cohort of paediatric CNS embryonal tumours and HGNETs. We demonstrated the utility DNA methylation arrays in diagnostics and outcome prediction.

Methods

Prior to 2019, paediatric patients with cancer in Hong Kong were managed in one of the five paediatric oncology units of the Hong Kong Paediatric Haematology/Oncology Study Group. We retrospectively identified patients with CNS embryonal tumours and associated diagnostic entities who were treated between 1999 and 2017. The relevant histologic entities included medulloblastoma, CNS-PNET, pineal parenchymal tumour, embryonal tumours with multi-layered rosettes (ETMR), embryonal tumour not otherwise specified, and HGNET. Patients with adequate archival tumour tissue were included.

Formalin-fixed paraffin-embedded (FFPE) tumour samples were retrieved, and DNA extraction was performed. Extracted DNA was quantified, and quality control was performed. DNA samples that met the quality control criteria (delta-Ct <5) underwent bisulphite conversion and were subsequently used for DNA methylation profiling targeting 850 000 CpG sites in the genome. The resulting raw Intensity Data files were analysed with the web-based DKFZ classifier (Molecular Neuropathology [MNP] 2.0 v11b4 https://www.molecularneuropathology.org/mnp), where each sample was assigned a molecular class and corresponding confidence score (Fig 1). In parallel, unsupervised t-distributed stochastic neighbour embedding (t-SNE) analysis was performed to compare tumours in this study with a publicly available CNS tumour reference cohort comprising 2801 samples.[3] Molecular classes were based on the results of both analyses and interpreted according to clinical context.



Methylation profiling report

General information

Sentrix ID:	204367400056_R06C01
Array type:	EPIC
Material type:	FFPE DNA
Gender:	male

Brain tumor methylation classifier results (v11b4)

Methylation classes (MCs with score >= 0.3)	Calibrated score	Interpretation
methylation class family Medulloblastoma group 3 and 4	0.99	match
MC family members with score >= 0.1		
methylation class medulloblastoma, subclass group 4	0.99	match

Legend: Match (score >= 0.9) No match (score < 0.9): possibly still relevant for low tumor content and low DNA quality cases. Match to MC family member (score >= 0.5)

Class descriptions

Methylation class family Medulloblastoma group 3 and 4: The methylation class family "Medulloblastoma class 3 and 4" comprises the methylation classes medulloblastoma, class 3 and medulloblastoma, class 4.

Methylation class medulloblastoma, subclass group 4: The methylation class "medulloblastoma, subclass group 4" is comprised of tumors with the diagnosis medulloblastoma, genetically defined, group 4. Histologically most cases fall into the classical and large cell/anaplastic groups. This is the most common molecular subtype of medulloblastoma (~35-40% of cases). Tumors originate in the cerebellum, typically in the vermis. Median age is 8 years (range 2-49 years). Group 4 medulloblastomas are more common in males than females. MYCN amplification, isochromosome 17q, and aneuploidy are recurrent features, but a fraction lack an obvious driving genetic change.

Copy number variation profile

Depiction of chromosome 1 to 22 (and X/Y if automatic prediction was successful). Gains/amplifications represent positive, losses negative deviations from the baseline. 29 brain tumor relevant gene regions are highlighted for easier assessment.
(see Hovestadt & Zapatka, http://www.bioconductor.org/packages/devel/bioc/html/conumee.html)

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FIG 1. A sample methylation profiling report

Overall survival was defined as the interval from diagnosis to date of death or final follow-up for survivors; progression-free survival was defined as the interval from the date of diagnosis to the date of the first event (disease progression, disease recurrence, second malignant neoplasm, or death from any cause) or final follow-up for patients without events. Survival estimates were reported using the Kaplan-Meier method. The log-rank test was used to compare outcomes among groups, and Cox regression was used for multivariate analysis. All P values were two-sided and considered statistically significant at <0.05.

Results

In total, 124 FFPE tumour samples were profiled by methylation arrays. Among these samples, 97 contained histologic CNS embryonal tumours/HGNETs, 16 contained histologic glioneuronal tumours that served as comparative controls, and 11 required repeat analysis due to technical issues

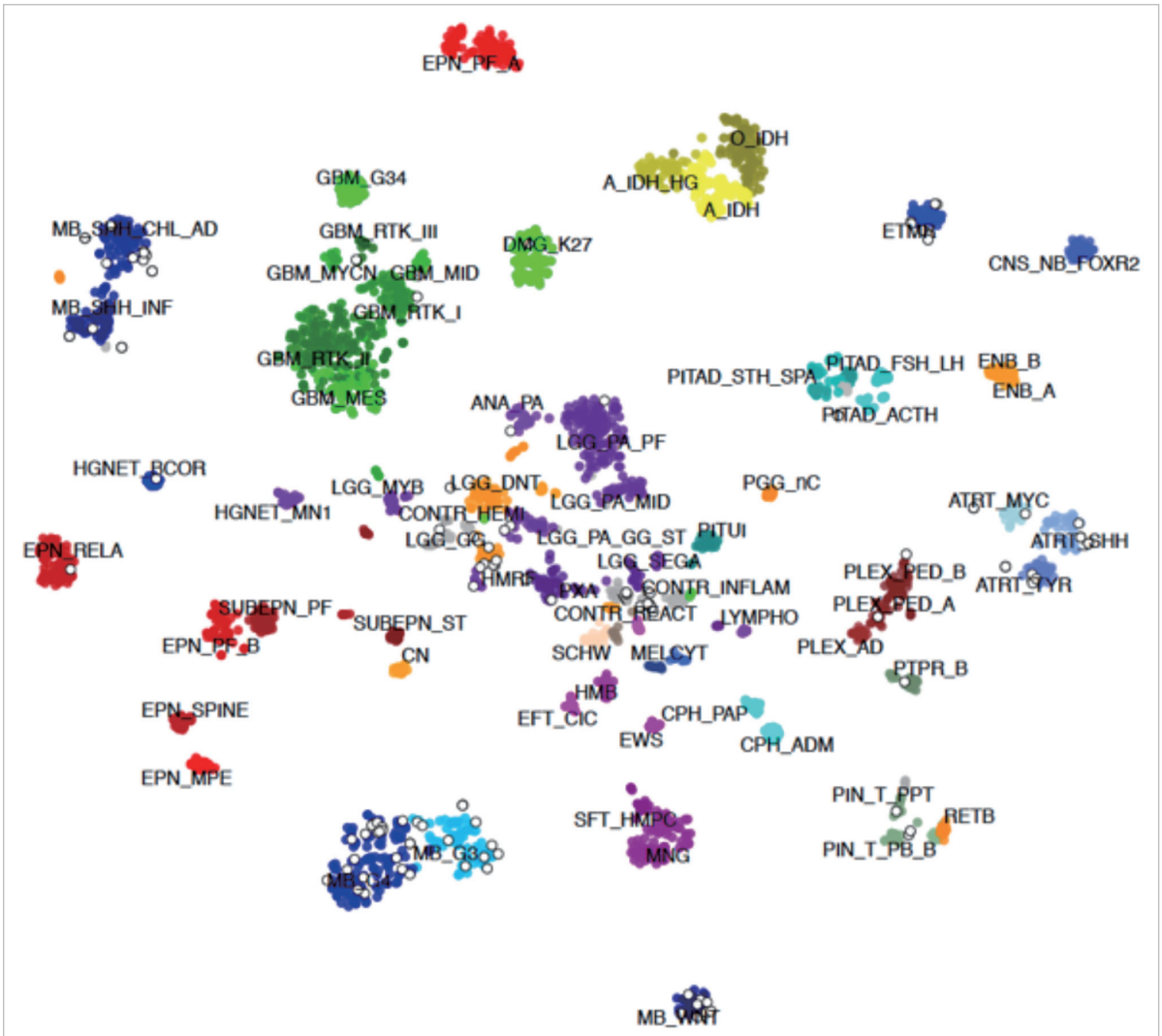

FIG 2. t-SNE analysis comparing the methylation profiles of our cohort (n=97, open white circles) and a publicly available central nervous system (CNS) tumour reference cohort (n=2801, coloured circles), indicating associations between samples and known molecular subclasses of CNS tumours with infrequent outliers

involving a defective array chip. We focused on the main cohort of CNS embryonal/high-grade neuroepithelial tumours.

Among the cohort of 97 samples, DNA was extracted from FFPE slides (n=75), scrolls (n=3), or a combination of the two (n=19). Seven samples required macrodissection to enrich tumour material because of low tumour content. The median quantity of total DNA extracted was 490 (range, 12.5-7290) ng. Up to 500 ng of DNA from each sample were submitted for methylation array analysis.

The cohort samples were from 59 male patients and 38 female patients. The median age at diagnosis was 5.7 (range, 0.6-22.2) years, and the median duration of follow-up was 4.6 (range, 0-20.6) years. Primary tumour locations were the cerebellar/posterior fossa (n=71), cerebral cortex (n=15), pineal gland (n=7), sellar/parasellar regions (n=2), ventricles (n=1), and spine (n=1). Metastasis was documented in 15% of the patients. Among patients with outcomes available (n=85), events occurred in 49; 47 events were tumour related.

The original clinicopathologic diagnoses included medulloblastoma (n=65), ATRT (n=9), pineal parenchymal tumour (n=8), ETMR (n=6), HGNET (n=4), CNS-PNET (n=4), and choroid plexus tumour (n=1). Random forest classifier assessment revealed calibrated scores of >0.9 (confident assignment) in 64 (65%) samples and >0.6 (confident and potentially relevant assignment) in 73 (75%) samples. Unsupervised t-SNE analysis based on a CNS tumour reference cohort (n=2801)[3] indicated that 85 (88%) samples had informative methylation profiles (Fig 2). The methylation-based

TABLE. Methylation-based assignment of central nervous system (CNS) embryonal tumours

Molecular entity	No. of samples (n=97)
Medulloblastoma	52
WNT-activated	8
SHH-activated (infant)	3
SHH-activated (children/adult)	10
Group 3	10
Group 4	21
Atypical teratoid/rhabdoid tumour	9
TYR	5
SHH	3
MYC	1
Pineal parenchymal tumour	6
Pineoblastoma	3
Pineal parenchymal tumour of intermediate differentiation	2
Papillary tumour of the pineal region	1
Embryonal tumour with multi-layered rosettes	7
Pituitary blastoma	2
High-grade neuroepithelial tumour, BCOR-altered	1
High-grade glioma	4
Others	4
Control/no match	12

assignments of samples are summarised in the Table.

Epigenomic profiling allowed molecular subgrouping and confirmation of diagnosis in 65 (67%) samples, confirmation of diagnosis in eight (8%) samples, and suggested alternative diagnosis in 12 (12%) samples. Among the remaining samples (n=12, 12%), four were molecularly similar to reference controls, suggesting non-neoplastic cell contamination, and eight did not cluster with any known references in the classifier or t-SNE analyses. Novel clinicopathologic-molecular associations were established, including an expanded clinical-molecular profile for the rare entity of pituitary blastoma.

Patient outcomes significantly differed according to molecular diagnoses. Patients with better prognosis included individuals with medulloblastoma in the WNT-activated, SHH-activated (children/adult subtype), and group 4 subgroups, which represented 'good-risk' entities. Patients with suboptimal outcomes included patients with medulloblastoma in the SHH-activated (infant subtype) and group 3 subgroups, ETMR, and high-grade glioma.

Discussion

Embryonal tumours, the most common CNS tumours in young children, constitute aggressive WHO Grade IV tumours with a tendency to metastasise. Recent epigenomic studies have revealed that various subtypes of CNS embryonal tumours (ie, medulloblastoma, CNS-PNET, pineoblastoma, and ATRT) are biologically distinct, with intertumoural heterogeneity within subtypes. The present study provides relevant data to support the use of methylation profiling for clinical management of children with CNS neoplasms in Hong Kong.

Molecular assays are often hindered by the difficulty of obtaining good-quality data based on FFPE-derived tumour DNA.[4] Our experience indicated that even when the quantity of DNA is suboptimal (<500 ng), there is value in proceeding with quantitative polymerase chain reaction–based quality control and downstream workflows. In our cohort, 88% of the samples were clustered with established tumour entities. Suboptimal classification is likely related to the predefined structure of current tumour classifiers, suggesting that success rates in methylation studies will continue to improve.

Paediatric patients with cancer in Hong Kong receive uniform treatment. Thus, the correlation between tumour classification and treatment outcomes allowed assessment of prognostic value in each molecular group. Medulloblastoma can be molecularly classified into WNT-activated, SHH-activated, group 3, and group 4 diseases.[5] The superior outcomes among patients with WNT-activated tumours and the aggressiveness of group 3 disease support treatment de-escalation and intensification, respectively. Among our cohort, 12% of samples were re-assigned to an alternative diagnosis. This percentage is similar to that in German and Dutch cohorts. Patients with tumours re-classified as high-grade gliomas had poor survival. Identification of novel entities/associations highlights the challenges in CNS tumour diagnostics based on existing pipelines.

Methylation arrays in tumour diagnostics are dependent on the quality, size, and complexity of the reference cohort with which study samples are compared. For example, CNS germ cell tumours are underrepresented in existing reference sets because of their low incidence in Western populations. To further enhance this epigenetics-based algorithm, multi-national collaborations and evaluations by expert neuropathologists should include integrated interpretation of clinical information, histomorphology, immunohistochemical profiles, and other targeted molecular studies.

Conclusion

DNA methylation profiling is useful in the diagnosis

of paediatric CNS embryonal tumours. Our cohort, one of the largest in Asia, provides a foundation for regional and international collaborations in paediatric neuro-oncology research.

Funding

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Disclosure

The results of this research have been previously published in:

1. Tam OCH, Ho RSL, Chan S, et al. Genome-wide DNA methylation profiling as frontline diagnostics for central nervous system embryonal tumors in Hong Kong. Cancers (Basel) 2023;15:4880.
2. Liu AP, Li KK, Chow C, et al. Expanding the clinical and molecular spectrum of pituitary blastoma. Acta Neuropathol 2022;143:415-7.
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4. Yang RR, Li KK, Liu APY, et al. Low-grade BRAF V600E mutant oligodendroglioma-like tumors of children may show EGFR and MET amplification. Brain Pathol 2021;31:211-4.

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