


DNA methylation microarray analysis of adult gliomas: A pilot study at Groote Schuur Hospital

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Background: Accurate glioma diagnosis requires a combination of histology, radiology and identification of key genetic mutations. Currently, multiple tests are required to identify these mutations. Deoxyribonucleic acid (DNA) methylation microarray coupled with digital classification algorithms can subclassify gliomas and identify multiple mutations in a single experiment, thereby potentially replacing current modalities and reduce turnaround times.

Aim: This study aims to compare results obtained by DNA methylation microarray on select adult glioma cases previously classified and graded on the basis of morphology and currently available ancillary tests.

Setting: Cape Town, South Africa.

Methods: Eight cases comprising astrocytic and oligodendroglial tumours (WHO grades 2–4) were analysed using the Illumina Infinium MethylationEPIC 850k microarray platform. Tumour classification and O⁶-methylguanine DNA-methyltransferase (MGMT) promoter methylation status were determined via online classification algorithms. Key genetic and chromosomal changes were identified by copy number variation plots.

Results: Seven of the eight cases were successfully assigned a methylation class and showed concordance with previously determined histological tumour type, isocitrate dehydrogenase and 1p/19q co-deletion status. Of these, tumour grading remained unchanged in five cases, upgraded in one case and downgraded in the other. The remaining case could not be classified. The MGMT promoter methylation status and diagnostically relevant copy number variants were also identified.

Conclusion: Tumour classification and grading can be accurately determined by methylation microarray analysis in adult gliomas.

Contribution: Methylation microarray provides greater molecular information than current methods, thereby potentially improving diagnostic accuracy and patient prognostication.

Keywords: methylation microarray; glioma; glioblastoma; IDH; MGMT.

Introduction

Adult gliomas encompass tumours demonstrating either astrocytic or oligodendroglial differentiation ranging from the World Health Organization (WHO) classification grades 2 to 4.^{1,2} Appropriate treatment of these tumours relies on accurate histological diagnosis to correctly establish cells of differentiation and determine tumour grade. The rapid development of molecular technology and its application to glioma pathology has resulted in the identification of many genetic markers that impact tumour classification and grading.^{2,3,4,5,6} This has resulted in a major shift in glioma diagnosis by histopathologists from a pure morphological diagnosis to one in which multiple additional genetic criteria are incorporated to ensure accurate diagnosis and assist with patient prognostication and predicted response to treatment.^{3,7}

The 2021 WHO classification of tumours of the central nervous system, 5th edition (WHO CNS 5) recommends, where possible, a final integrated diagnosis that incorporates histological diagnosis with additional supportive molecular information.⁸ In order to render an integrated diagnosis, a combination of immunohistochemistry (IHC), Deoxyribonucleic acid (DNA) sequencing and fluorescence *in situ* hybridisation (FISH) studies are ideally required.³ In certain settings, however, depending on the setup of the laboratory, the turnaround time may be prolonged, and tissue may be sent to multiple specialist centres for analysis; additionally, each test requires a subminimal amount of viable tumour DNA from limited tissue.

An example of integrated diagnosis is shown further:

- Integrated diagnosis: Diffuse astrocytic glioma, Isocitrate dehydrogenase (IDH)-wildtype, with molecular features of glioblastoma WHO 4
- Histological diagnosis: Anaplastic astrocytoma
- Molecular information: IDH wildtype (sequencing IDH1 and IDH2), chromosome +7/−10 (FISH), Epidermal growth factor receptor (EGFR) amplified (FISH)

In recent years, DNA methylation microarray technology has gained momentum in the diagnosis, classification and grading of gliomas because of differential genome-wide epigenetic (methylation) patterns found in these tumours.^{9,10,11} There are a variety of methods that could be used to analyse DNA methylation, each with varying applications in oncology.¹² One such platform, the Illumina Infinium MethylationEPIC BeadChip 850k microarray, utilises fluorescent-tagged DNA probes that target approximately 850000 methylation-sensitive DNA sequences known as cytosine-guanine dinucleotide (CpG) islands.^{12,13,14}

Gene promoter and enhancer regions are enriched for CpG islands and DNA methylation primarily occurs at the carbon-5 position of CpG cytosines termed as 5-methylcytosine (5mC).¹⁵ In cancer, global CpG methylation is observed thereby resulting in promoter site methylation and reduced gene transcription, most importantly of tumour suppressor genes.¹⁶ There are also cell type-specific patterns of CpG methylation that may persist in tumour development and progression.¹⁷ This underlies the increased need for the integration of DNA methylation profiling in the classification of CNS tumours.

Briefly, upon incubation of target genomic DNA with sodium bisulfite, non-methylated cytosine residues are chemically converted into uracil while 5mC remains unchanged. Fluorescent-labelled methylation-specific probes corresponding to CpG islands are annealed to the samples and are able to discriminate between methylated and unmethylated CpG islands across the genome. This results in a genome-wide methylation 'signature' for the tumour or the CpG island methylation phenotype (CIMP).¹⁴

Capper et al.⁹ analysed 2801 tumours (representing all CNS tumours, not exclusively gliomas) by using methylation microarray and identified 82 distinct classes. This resulted in the development of the so-called 'Heidelberg Classifier' where raw output data (IDAT files) from methylation studies can be uploaded to a secure remote server and the diagnosis confirmed by a classifier. With the Heidelberg classifier, methylation microarray analysis can simultaneously determine:

- IDH mutational status. Inferred by whole genome methylation pattern (CIMP).⁵ This is detected irrespective of whether mutation is the most common IDH1 R132H, non-R132H or IDH2 mutations.
- 1p/19q co-deletion status; this is essential for the diagnosis of oligodendroglioma.⁵

- Copy number variations (amplifications and deletions), for example, EGFR
- Other chromosomal changes (gains or deletions), for example, gain of chromosome 7 and loss of chromosome 10 (+7/−10)
- O⁶-methylguanine DNA-methyltransferase (MGMT) promoter methylation status. This relies on an additional algorithm (MGMT-STP27) and shows high concordance with MGMT pyrosequencing.^{9,18}

Currently, to achieve this, a combination of multiple platforms including IHC, FISH, polymerase chain reaction and DNA sequencing are required, usually performed in specialist centres, resulting in delays in diagnosis. Methylation microarray, therefore, has the potential to maximise information that can be extracted from limited tissue samples, improve turnaround times and provide treating oncologists with an accurate, comprehensive and integrated diagnosis.

Aim of the current study

This pilot study aimed to compare the results obtained from methylation microarray with those obtained by morphology and locally available ancillary tests from select adult gliomas encompassing a range of WHO grades (2–4) for astrocytic and oligodendroglial tumours.

Methods

Scientific design

The study was retrospective in nature. Cases were obtained from the National Health Laboratory Service (NHLS) TrakCare information system using appropriate Systematised Nomenclature of Medicine (SNOMED) codes. The period 2016–2020 was chosen in order to maximise the probability of optimal DNA integrity as older formalin-fixed paraffin-embedded (FFPE) tumour blocks may show excessive DNA fragmentation. This was also the period during which routine IDH1 R132H testing by IHC was introduced in the division.

Inclusion criteria

Patients over 18 years of age, primary gliomas including diffuse astrocytoma, oligodendroglioma and glioblastoma were included. All cases required IDH1 R132H IHC and additional 1p/19q co-deletion FISH results for cases diagnosed as oligodendroglioma. Cases for methylation microarray were chosen to represent both IDH mutant and wildtype astrocytic and oligodendroglial tumours for WHO grades 2–4 to assess the ability of the methylation classifier to identify key discriminating mutations.

Exclusion criteria

Paediatric cases, samples with limited tumour (tumour occupying < 30% total surface area), samples with significant necrosis and those for which IHC and /or FISH results were not available. Other exclusions included benign CNS neoplasms, non-glioma neoplasms and metastatic tumours.

Cases were reviewed in terms of morphology, immunohistochemical (IHC) profile and final diagnosis. All tumours that were IDH R132H negative by IHC were labelled not otherwise specified (NOS), per guidelines of WHO as they were not sequenced for identification of non-canonical IDH1/2 mutations.⁵ In the case of patients older than 55 years with tumours exhibiting high-grade features in keeping with glioblastoma but negative IDH1 stains, the WHO recommends that the designation of wildtype be assigned as the risk of non-IDH1 mutations are less than 1%.⁵ However, these cases were assigned as NOS if additional clinical/radiological information was not readily available. The MGMT promoter methylation testing is currently not offered in the division. The final tumour histological terminology incorporates the latest WHO CNS 5 tumour classification terminology.^{2,5}

Immunohistochemistry and fluorescence *in situ* hybridisation

Isocitrate dehydrogenase mutational status was determined on FFPE using mouse monoclonal IDH1 R132H antibody (clone ICH132) (GenomeMe, Richmond, Canada) and enzyme-linked secondary antibody. Cases that were both IDH1 R132H positive and morphologically consistent with oligodendroglioma or cases showing focal areas suggestive of oligodendroglial differentiation were sent for 1p/19q co-deletion FISH (NHLS, Johannesburg).

DNA methylation microarray

One FFPE block was chosen for each case and contained regions with greatest tumour burden. For each case, 10 × 10 micrometre unstained sections were collected onto glass slides and dried on a heat plate. One haematoxylin and eosin-stained slide was also included. Samples, together with histology reports and export permit, were couriered to the Department of Neuropathology, University College London, London, United Kingdom. Upon arrival, the cases were reviewed by an expert neuropathologist. Where necessary, the tumour was micro-dissected from surrounding tissue. DNA was extracted and bisulfite converted prior to quality control analysis. The samples were analysed by Illumina Infinium MethylationEPIC 850k microarray.

Methylation data analysis

Raw IDAT files generated by the microarray were uploaded to a secure server (<http://www.molecularneuropathology.org>). Brain tumour classifier 11b4 (Version 3.0) was used. Calibrated scores were given for methylation class and subclass for each sample. A class-calibrated score ≥ 0.90 indicates good DNA quality and reliable test results. A subclass calibration score ≥ 0.5 indicates match to the methylation class family member. The MGMT promoter methylation status was determined using the MGMT-STP27 logistic regression algorithm.¹⁹

The Heidelberg classifier provides three main outputs: (1) the tumour class based on the CIMP, which includes IDH

mutational status by inference, (2) Copy number variation (CNV) plots of chromosomes and specifically 29 tumour-associated genes and (3) MGMT promoter methylation status. The cancer genome atlas (TCGA) glioma classifier was used for one case that could not be classified by the Heidelberg algorithm.²⁰

Ethical considerations

Ethical clearance to conduct this study was obtained from the University of Cape Town, Faculty of Health Sciences Research Ethics Committee (HREC 817/2020).

Results

Study population and demographics

The sample group comprised five males and three females. The age range was 21–68 years (Table 1). Case three had a history of a prior anaplastic astrocytoma resection and case four had previous neuroimaging suggestive of low-grade glioma but was lost to follow-up. Case eight had a reported family history of neurofibromatosis and a histological diagnosis of breast neurofibroma; however, no additional information was provided.

Tumour classification and grading

Six out of the eight samples assigned to a methylation class via the Heidelberg classifier were concordant with the histological diagnoses (Table 1).

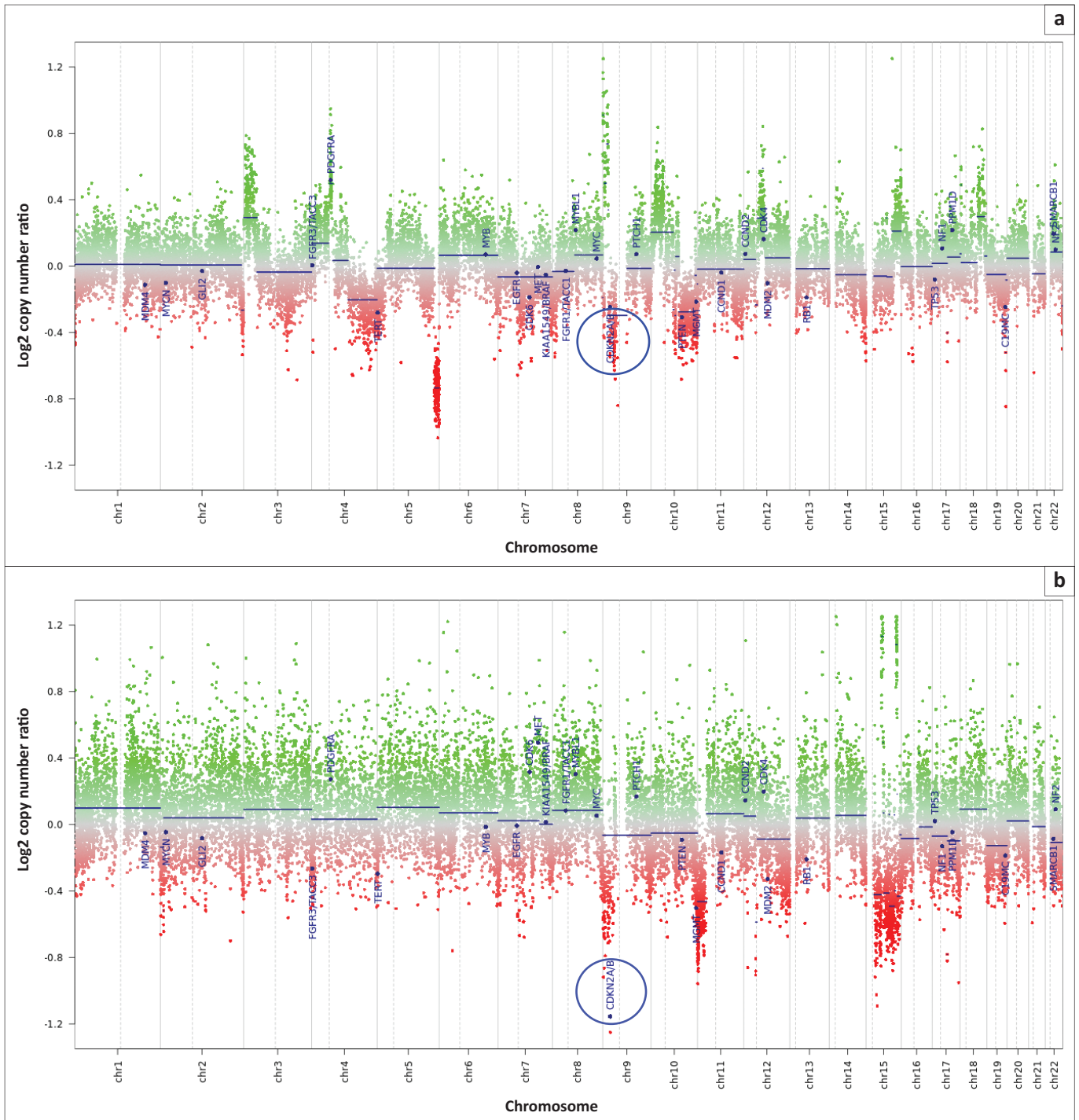
All cases designated astrocytoma by histology were confirmed as such by the classifier (cases one, three, six). Case one was, however, upgraded from astrocytoma IDH mutant WHO grade 2 to grade 3. The higher grade of the tumour was established by the presence of high copy number variations (Figure 1a). As no homozygous deletion of cyclin-dependent kinase inhibitor 2A/B (CDKN2A/B) was present, this profile is consistent with WHO grade 3 versus grade 4. Case three remained unchanged as grade 3 astrocytoma and in case six, grade 4 status was confirmed with homozygous CDKN2A/B loss (Figure 1b). Isocitrate dehydrogenase mutational status was unchanged in cases one and six positively identified by IHC. Case three, designated IDH NOS, was determined to be IDH mutated by the classifier. As this case was negative by IHC, it may indicate either a less common non-IDH1 R132H mutation or IDH2 mutation.

Oligodendroglioma diagnosis was confirmed in both cases four and five by means of demonstration of loss of whole arms of 1p and 19q (1p/19q co-deletion) (Figure 2). Isocitrate dehydrogenase mutational status was inferred by genome-wide methylation status. Case five was downgraded from anaplastic oligodendroglioma (WHO grade 3) to oligodendroglioma (WHO grade 2) by the classifier. Further review of the histological slides reveals no increased mitotic figures; however, focal areas of increased vasculature were interpreted as microvascular

TABLE 1: Summary of case demographics with comparison of conventional histological diagnosis with methylation profiling.

Case number	Age at diagnosis	Gender	Tumour location	Additional relevant clinical information	IDH1 R132H status (IHC)	1p/19q co-deletion status (FISH)	Initial diagnosis	Methylation classifier	MGMT promoter status	Grade change (upgrade/downgrade/unchanged)	IDH status change?
1	43	Male	Left frontal and temporal lobes	Not applicable	Positive	Not applicable	Astrocytoma, IDH-mutant (WHO grade 2)	IDH glioma, subclass high grade astrocytoma (WHO Grade 3)	Methylated	Upgrade	No
2	60	Female	Midline callosal	Not applicable	Negative	Not applicable	Astrocytoma, NOS (WHO grade 2)	Control tissue, tumour microenvironment	Unmethylated	Not applicable	Not applicable
3	31	Male	Left frontal lobe	Previous resection 4 years prior. Previously diagnosed anaplastic astrocytoma	Negative	Not applicable	Anaplastic astrocytoma, NOS (WHO grade 3)	IDH glioma, subclass high grade astrocytoma (WHO Grade 3)	Methylated	Unchanged	Yes, NOS -> mutant. May represent non-R132H IDH1 mutant or IDH2 mutant
4	49	Female	Right frontal lobe	2011 imaging suggestive low grade glioma, lost to follow up	Positive	Present	Oligodendroglioma, IDH-mutant and 1p/19q co-deleted (WHO grade 2)	IDH glioma, subclass 1p/19q co-deleted oligodendroglioma (WHO grade 2)	Methylated	Unchanged	No
5	67	Male	Frontal lobes bilateral	Not applicable	Positive	Present	Anaplastic oligodendroglioma, IDH-mutant and 1p/19q co-deleted (WHO grade 3)	IDH glioma, subclass 1p/19q co-deleted oligodendroglioma (WHO grade 2)	Methylated	Downgrade	No
6	35	Male	Left frontal lobe	Not applicable	Positive	Not applicable	Astrocytoma, IDH-mutant (WHO grade 4)	IDH glioma, subclass high grade astrocytoma (WHO grade 4)	Methylated	Unchanged	No
7	68	Male	Right occipital lobe	Not applicable	Negative	Not applicable	Glioblastoma, NOS (WHO grade 4)	Glioblastoma, IDH-wildtype, subclass RTK II (WHO grade 4)	Methylated	Unchanged	Yes, NOS -> wildtype
8	21	Female	Right frontal lobe	Family history of neurofibromatosis. Breast neurofibroma	Negative	Not applicable	Glioblastoma, NOS (WHO grade 4)	No matching methylation class via methylation classifier. Tumour classified as LGM6 via TCGA classifier	Methylated	Not applicable however morphologically high grade astrocytoma	No

IHC, immunohistochemistry; FISH, fluorescence *in situ* hybridisation; IDH, isocitrate dehydrogenase; WHO, World Health Organization; RTK, receptor tyrosine kinase; NOS, not otherwise specified; MGMT, O⁶-methylguanine DNA-methyltransferase.



Note: Plot Numbers (a) 20564800030_R02C01 and (b) 20564800030-R05C01 both refer to the anonymised codes used for the samples.

FIGURE 1: Copy number variation plot of high-grade glioma, IDH mutant. Gains and/or amplifications are depicted as positive (green) deviations from baseline (zero) and losses as negative (red) deviations. Three cases were assigned to this category which encompass astrocytoma, IDH mutant grades 3 and 4. Figure 1(a) Case three shows decreased levels of CDKN2A/B (blue circle), in keeping with astrocytoma IDH mutant grade 3. Figure 1(b) In contrast, case six shows homozygous CDKN2A/B loss (blue circle) in keeping with astrocytoma IDH mutant grade 4. Case 1 showed features similar to case three (result not shown). Additional brain tumour relevant genes (total of 29) are automatically highlighted as part of the Heidelberg classifier output.

proliferation on the original report. These areas are most likely not true microvascular proliferation and the methylation classifier diagnosis was considered to be a true reflection of the tumour grade.

Case seven was confirmed as a glioblastoma by the classifier with additional assignment to receptor tyrosine kinase (RTK) II subclass. Isocitrate dehydrogenase wildtype status was confirmed. This case was initially designated NOS as a result

of negative R132H immunohistochemical staining. The CNV plots additionally demonstrated EGFR amplification and +7/-10, both features diagnostic of glioblastoma²¹ (Figure 3).

Two cases could not be successfully classified by the Heidelberg algorithm. Case two, morphologically a diffusely infiltrative low-grade diffuse glioma, was assigned to the 'control tissue, reactive tumour environment' class. This methylation class is usually assigned to cases with low

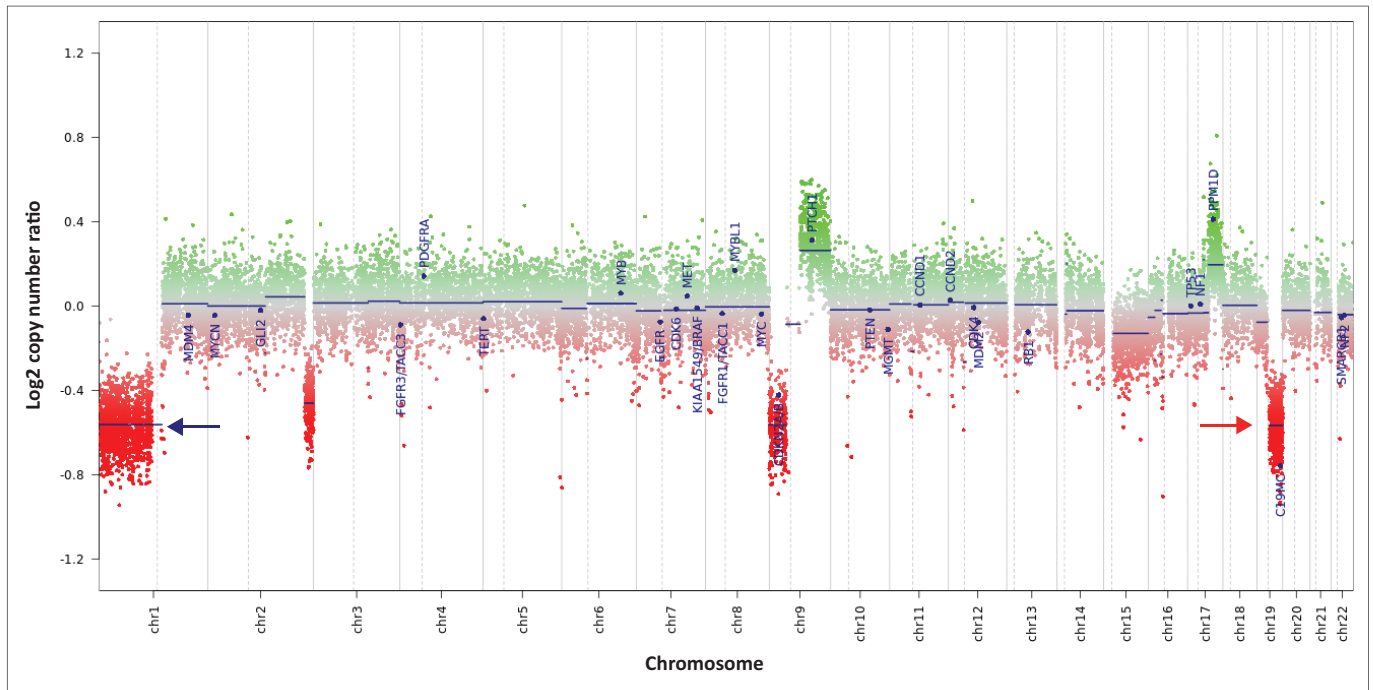


FIGURE 2: Copy number variation plot of oligodendroglioma, IDH mutant and 1p/19q co-deleted, for chromosomes 1 to 22. Gains/amplifications depicted as positive (green) deviations from baseline (zero) and losses as negative (red) deviations. Cases four and five showed similar features; only case four is shown here. Chromosome 1 shows deletion of the short arm (blue arrow). Chromosome 19 demonstrates deletion of the long arm (red arrow). Note that probes span entire length of chromosome arms – an advantage over FISH; centromeric regions are not included.

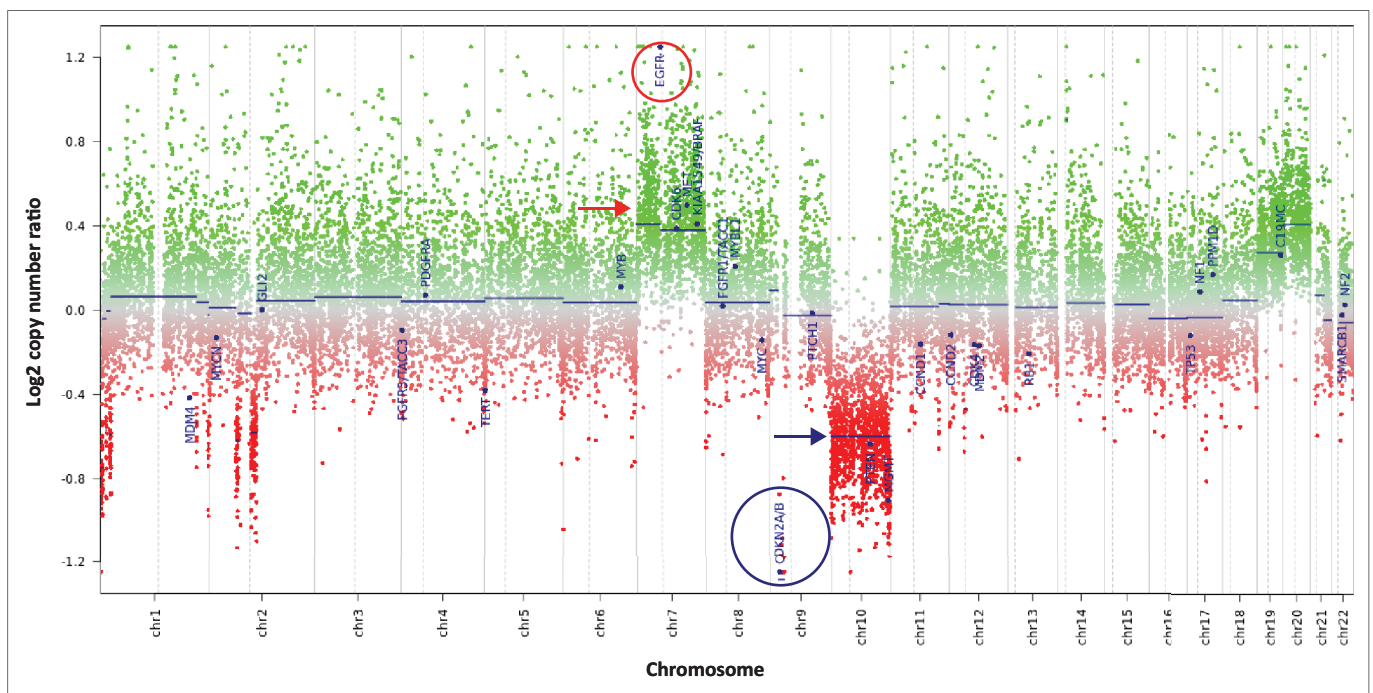


FIGURE 3: Copy number variation plot of glioblastoma, IDH wildtype, for chromosomes 1 to 22. Gains/amplifications depicted as positive (green) deviations from baseline (zero) and losses as negative (red) deviations. Loss of chromosome 10 is indicated by a blue arrow, gain of chromosome 7 by a red arrow. Chromosome 7 shows amplification of EGFR (red circle). Chromosome 9 shows CDKN2A/B homozygous deletion (blue circle).

tumour content in relation to surrounding reactive, non-neoplastic tissue (Figure 4a).

For case eight, despite histological features of a grade 4 tumour (prominent microvascular proliferations) and multiple copy number variations, no methylation class could

be accurately assigned (Figure 4b). Of note is the family history of neurofibromatosis. In order to explore whether a methylation class could be obtained with a different algorithm, the raw methylation IDAT files of this case were uploaded and passed through the TCGA glioma classifier via TCGAbiolinksGUI. The classifier assigned this case to the

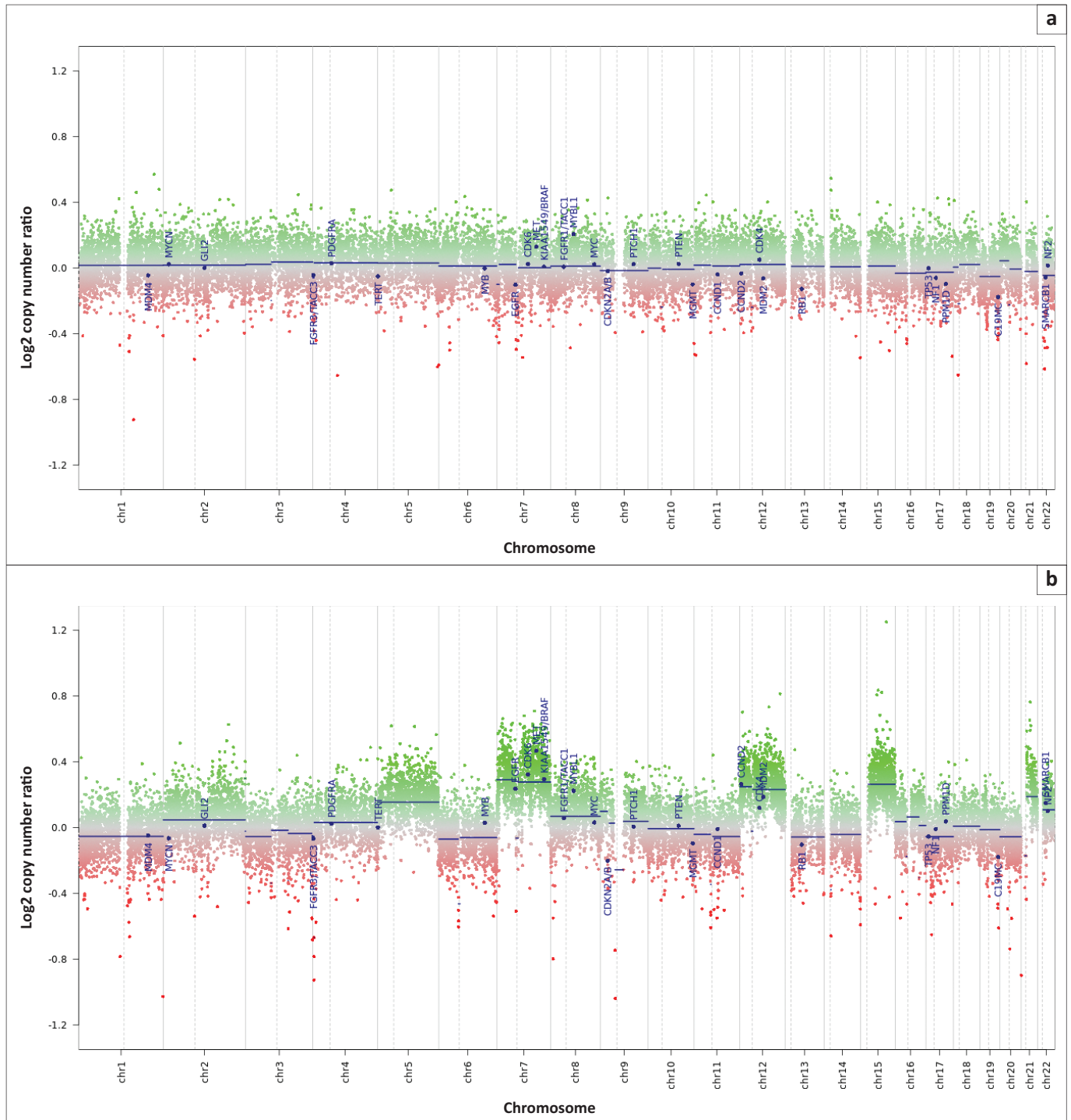


FIGURE 4: Cases for which no class could be assigned via the Heidelberg classifier. Gains and/or amplifications are depicted as positive (green) deviations from baseline (zero) and losses as negative (red) deviations. (a), Case two. Histologically graded as diffuse astrocytoma WHO grade 2. Classifier determined as reactive tumour microenvironment. Cases belonging to this class show low tumour cell content and copy number changes may be masked by abundant background non-neoplastic areas. The copy number plot shows a 'flat' pattern. (b), Case eight. The histological features are that of a high-grade glioma and the copy number profile indicates multiple chromosomal gains (green) and losses (red).

LGM6 group of tumours which are IDH wildtype.²² Neurofibromatosis-1 associated gliomas have previously been described as belonging to this methylation class.²³

All cases that were successfully classified demonstrated MGMT promoter methylation. The unmethylated result obtained for case 2 may actually reflect the large amounts of background non-neoplastic glial tissue and may not be representative of the tumour.

Discussion

This study is the first to investigate DNA methylation microarray technology for the classification and grading of adult glial tumours at our institution. To this end, the study has demonstrated that adequate amounts of high-quality DNA can be retrieved from archival FFPE specimens, bisulphite converted, and analysed by the microarray platform.

Agreement between tumour diagnosis and grading was achieved in the majority of cases. The cases where the grade was changed highlight key challenges frequently encountered in neuropathology. In case one where astrocytoma grade was changed from 2 to 3 by the methylation classifier, this was made on the basis of detection of key molecular changes that would not be visible microscopically, namely gene copy number variations. Additionally, as tumour biopsies are usually small, there is the risk that the portion of tumour sampled may not show features indicative of higher grade (in this case, mitotic figures) but still carry molecular signatures in keeping with more aggressive behaviour. Determination of the signatures would ensure that patients are not undertreated. In case five, where a grade 3 oligodendroglioma was changed to grade 2 by the classifier, review of the histological slides revealed that unequivocal microvascular proliferation was, in fact, not present. This highlights the degree of interobserver variability in neuropathology.

Isocitrate dehydrogenase mutational status plays an important role in glioma prognosis with patients having significantly better overall survival in patients with IDH-mutant tumours compared to wildtype.⁵ One case identified as NOS was changed to IDH mutant by methylation array (case three). According to WHO guidelines, tumours that are negative for IDH1 R132H by IHC staining should, ideally, be sequenced to identify non-R132H IDH1 mutations (codon 132, including R132C, R132S, R132L) and IDH2 mutations (codon 172).⁵ If this is not available, the tumours are designated NOS instead of wildtype. Confirmation of IDH wildtype status by methylation would allow for the correct prognostication of the patient. Isocitrate dehydrogenase gene sequencing is not currently offered in our division and therefore determination of all possible IDH1 and 2 mutations by means of genome-wide CIMP using methylation microarray is a significant advantage.

In addition to providing a histological classification by means of methylation profiling, the microarray platform successfully determined key copy number variations that serve to both confirm diagnosis (e.g. 1p/19q co-deletion in oligodendroglioma) and flag key genes/chromosomes for molecular grading (e.g. homozygous deletion of CDKN2A/B, EGFR amplification and +7/-10 for glioblastoma). Finally, the MGMT-STP27 algorithm successfully determined MGMT promoter methylation status in all successfully classified tumours which is helpful for the treating oncologists considering temozolomide. Previous reports indicate that MGMT promoter methylation status determination by STP-27 algorithm is a reliable alternative to PCR-based methods.¹⁸

The two cases that were not successfully classified by the Heidelberg algorithm provide important insights into some limitations of this technology. Case two was diagnosed as grade 2 diffuse astrocytoma on the basis of morphology both locally and during review by a neuropathologist prior to methylation microarray.

The high degree of surrounding non-neoplastic glial tissue, however, did not allow for the correct methylation profiling to occur and this case was assigned to the tumour control microenvironment category. This 'dilution' of tumours exhibiting significantly permeative growth patterns by surrounding non-neoplastic tissue must be borne in mind when considering methylation analysis. In these cases, careful microdissection may be required. Ultimately, the final diagnosis was made by histology alone, and this also echoes previous recommendations that methylation microarray be interpreted in conjunction with histology and not be considered a replacement for morphological analysis.³ Case eight was diagnosed as a glioblastoma (WHO 4) by histology but could not be assigned to a distinct methylation class via the Heidelberg algorithm. Though the high copy number variations were in keeping with high-grade behaviour, it was hypothesised that the family history of neurofibromatosis might account for unusual methylation profiles. The methylation data for case eight were passed through the TCGAblinks algorithm and were successfully assigned to a methylation class called LGM6.²² Neurofibromatosis-associated gliomas have previously been shown to belong to this class.²³ These tumours are IDH wildtype and span WHO grades 2-4. The Heidelberg methylation classifier may therefore be less useful in the analysis of tumours that are associated with a familial syndrome⁹ or in cases of lower-grade diffuse gliomas.

Practically and logistically, the use of a single platform for the identification of multiple key diagnostic and prognostic molecular parameters makes methylation microarray an attractive option. Additionally, less sample is required as all these parameters are determined simultaneously from methylation probe data produced in one run. The potential for improved turn-around time is great and would allow patients to receive a comprehensive molecular diagnosis more quickly and start treatment earlier.

Strengths and limitations of the study

Strengths

This is the first local study investigating the application of DNA methylation microarray technology in adult gliomas. The results confirm that many important diagnostic and prognostic features can be obtained from DNA-extracted FFPE tissue, thereby circumventing the need for fresh, unfixed tissue. With a single platform, diagnostic whole-genome DNA methylation patterns can be determined and classified together with the detection of important gene and chromosomal copy number variations.

Limitations

The number of cases investigated in this study is low. Because of the cost of each sample, only a few select cases were chosen but further investigation of this technology to other CNS

tumours, both non-glial and benign should be explored. Cost concerns are real, and in our setting, this may be the main limiting factor. At the current exchange rate of \pm R24.00/British pound, each microarray sample costs in the region of R10 800.00. However, as other molecular biology technology costs continue to decrease (viz., sequencing, PCR, etc.), one might expect that this would follow suit in the future. Additionally, the advantage of greater molecular information with resultant more accurate patient prognostication may result in cost benefits in ensuring that patients are appropriately treated. Going forward, methylation profiling would ideally be performed locally; this could be outsourced to local service providers and ultimately expansion of cases tested would assist in motivation for acquiring the platform at our institution.

Another key limitation of microarray analysis is that certain genetic point mutations and gene fusions cannot be detected. Examples include TP53 mutations, BRAF V600E and TERT promoter mutations. Though these parameters may be useful to know, the key diagnostic and prognostic parameters are addressed by the microarray. IHC stains are also available for these gene mutations and can be readily determined using this method if required.

Practise implications

The use of DNA methylation technology holds promise to improve turnaround times and reduce interobserver variability while identifying molecular signatures that may portend more aggressive behaviour. The determination of IDH status and MGMT promoter methylation status are two key features that will be of immediate use to treating neuro-oncologists. A single platform would also allow for conservation of precious, often limited, brain biopsy material. With as little as 250 ng of DNA, the entire process can be completed within 4 days.¹¹ Because of higher costs, methylation could initially be reserved for diagnostically challenging cases.

Current recommendations are for the integration of DNA methylation results with histological diagnosis to reach a consensus diagnosis. Therefore, DNA methylation results should not be interpreted in isolation. Importantly, it is worthwhile to note that methylation profiling by classification algorithms is currently considered research tools and under development and have not been clinically validated.

DNA methylation classification has also been applied to other CNS tumours, including meningiomas and paediatric tumours.^{24,25} Non-CNS tumours have also been analysed with methylation microarray including sarcoma.²⁶ In an autopsy study of carcinoma of unknown primary origin, methylation classifier could correctly predict origin in 87% of cases.²⁷ As the number of methylation-specific probes in the microarray are standard, a single run could be performed on multiple tumour types.

Conclusion

This pilot study is a proof of the concept that demonstrated successful methylation analysis of bisulfite-converted FFPE DNA obtained from archival adult glioma specimens.

An integrated diagnosis incorporating morphology and molecular information would be ideally suited to limited tissue, tissue with crush effect, or in cases where histological grade is discordant with radiological or intra-operative surgical findings.

This technology has the potential to provide more key molecular information with less tissue and faster turnaround times compared to current platforms.

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Competing interests

The author has declared that no competing interest exists.

Author's contributions

B.P. is the sole author of this research article.

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Data availability

Raw data were generated at the University College London and downloaded in IDAT format. Derived data supporting the findings of this study are available from the corresponding author, B.P., on request.

Disclaimer

The views and opinions expressed in this article are those of the author and do not necessarily reflect the official policy or position of any affiliated agency of the author, and the publisher.

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