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ORIGINAL PAPER

Adult grade II diffuse astrocytomas are genetically distinct from and more aggressive than their paediatric counterparts

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Abstract Diffuse astrocytomas (WHO grade II) typically present as slow-growing tumours showing significant cellular differentiation, but possessing a tendency towards malignant progression. They account for ~10% of all astrocytic tumours, with a peak incidence between 30 and 40 years of age. Median survival is reported as around 6–8 years. Mutations of *TP53* and *IDH1* have been described as genetic hallmarks, while copy number alterations are also relatively common. However, there is some evidence to suggest that these characteristics may vary with age. Here, we present an integrated clinicopathologic, genomic and transcriptomic analysis suggesting that paediatric and adult tumours are associated with distinct genetic signatures. For example, no childhood tumour showed mutation of *IDH1/2* or *TP53*, virtually no copy number changes were seen, and *MGMT* methylation was absent. In contrast, adult tumours showed *IDH1/2* mutation in 94% and *TP53* mutation in 69% of cases, with multiple copy number alterations per case and hypermethylation of *MGMT* in the majority of tumours. These differences were associated with a worse prognosis in the adult patients. The expression array data also revealed a significant difference in the expression of a number of genes putatively involved in neural stem cell maintenance and CNS development, including *DLL3, HES5, BMP2, TIMP1* and *BAMBI*. Genes involved in DNA replication and the cell cycle were also enriched in the adult tumours, suggesting that their more aggressive behaviour may be due to derivation from a more rapidly dividing, less differentiated cell type.

Keywords Astrocytoma · Paediatric · Differentiation · TP53 · IDH · MGMT

Introduction

Diffuse astrocytomas (WHO grade II, AII) are typically slow growing and display a marked degree of differentiation. They do, however, diffusely infiltrate the brain parenchyma, and in contrast to pilocytic astrocytomas (with which they are often grouped under the umbrella 'low-grade astrocytoma') they show an intrinsic tendency towards malignant progression [18]. Peak incidence of AII is in adults, with a mean age of ~41 years, and they account for 5–10% of astrocytic tumours [24, 25]. The overall survival times show significant variation between cases, with a median of around 6 years [24]. However, there is evidence that this may vary with age, with one report of 10-year survival rates of 81 and 39%, respectively, for age groups 0–19 and 20–44 years [4].

Mutations in *TP53* and more recently in *IDH1/2* have been reported as genetic hallmarks of grade II diffuse

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astrocytomas, with each occurring in around 50–80% of cases [2, 12, 25]. In addition, there is evidence in the literature of variability of mutation rates with age, with dramatically lower frequencies of *TP53* mutation in paediatric as opposed to adult patients [7]. This is also the case with *IDH1/2* mutations, although there is also an overriding trend for mutations to occur with younger age at diagnosis [8]. Recurrent copy number changes on 7q, 8q, 9 and 19 have also been observed [1, 9].

This study presents an integrated analysis of clinicopathological, genetic, copy number and expression data in a series of diffuse astrocytoma grade II ($n = 21$) including five paediatric patients. Very pronounced differences were seen in mutation rates of *IDH1/2* and *TP53* with age, as well as in frequency of copy number aberration and prognosis. Adult tumours also showed higher levels of methylation and concomitant reduced expression of *MGMT*. Finally, we show significant differences in the expression of a number of genes with suggested roles in neural stem cell self-renewal and CNS differentiation, suggesting that the behaviour of the adult tumours might in part be explained by a more stem-like phenotype. Together, these data suggest that adult and paediatric AIIs are distinct entities characterised by divergent molecular genetic profiles and behaviour.

Materials and methods

Patients, tumour tissue and nucleic acid extraction

Primary tumour samples from 21 patients with diffuse astrocytoma (WHO grade II) were included in the analysis. Five cases were from paediatric patients (<18 years), and 16 from adults. Age at operation ranged from 6 to 52 years (median 29 years). Histopathological classification was according to the most recent WHO recommendations [18]. The tumours were resected at the Karolinska Hospital, Stockholm and the Sahlgrenska University Hospital, Gothenburg, Sweden, between 1987 and 1997. All tumour pieces selected for nucleic acid extraction had high tumour cell content (minimum of 70%, generally >90%) as estimated by histological examination. DNA was extracted from tumour pieces as described previously [11]. RNA was extracted and cleaned with an RNeasy® kit (Qiagen) according to manufacturer's protocols. Patients and tumours, including histological subtyping where possible, are described in Table 1.

Microarray comparative genomic hybridisation

Construction of our 1-Mb whole-genome microarray as well as labelling, hybridisation and analysis protocols have all been described previously [19]. The raw data for the present series have been deposited in the Gene Expression Omnibus (GEO) with accession number GSE23869.

Mutation screening

Exon 4 of the *IDH1* gene was amplified and sequenced as previously described [12, 26]. Primers for *IDH2* exon 4 were according to Parsons et al. All previously described *IDH1/2* mutations have occurred in this exon.

The whole coding region and flanking intronic sequences of *TP53* were assessed for mutations through denaturing gradient gel electrophoresis (DGGE) and subsequent confirmation by direct sequencing, as previously described [11].

MGMT promoter region methylation analysis

Bisulphite DNA preparation, PCR reactions and pyrosequencing analysis were essentially as previously described [16], with primers as detailed in Supplementary Table 1.

Quantitative RT-PCR

A LightCycler® 480 Real-Time PCR System (Roche Diagnostics Ltd, Burgess Hill, UK) was used with SYBR Green I chemistry to evaluate expression levels of target genes. Primer sequences and cycling conditions are listed in Supplementary Table 1. Samples A6, A12, A27 and A40 did not have sufficient quality RNA for analysis. For the remaining 17 samples, the *TBP* gene (TATA binding protein) was used as a housekeeping control for relative quantification as calculated using LightCycler 480 software version 1.2 (Roche Diagnostics Ltd, Burgess Hill, UK). Samples were run in duplicate and a negative (no template) control was included in each assay.

Expression microarray analysis

Global expression profiles of a subset of tumours (5 adult and 5 paediatric) were determined using an Illumina HumanHT-12 v3 BeadChip (Illumina, Inc. San Diego, CA, USA). RNA amplification and labelling, array hybridisation and data extraction were performed by Cambridge Genomic Services (University of Cambridge, UK). RNA with RIN > 6 as assessed using an Agilent 2100 Bioanalyzer (Agilent Technologies UK Ltd., Wokingham, UK) were included. Data analysis was performed using the lumi and limma packages in R [5, 29, 30]. Probes scored as absent (detection $p > 0.01$) in all cases were excluded, leaving 17,096 probes for downstream analysis. The expression levels were transformed using variance stabilisation and normalised with a quantile normalisation (lumi

Table 1 Patient and tumour characteristics

ID	Sex	Location	Age	Histology	1 Mb Gains	1 Mb Losses	IDH1/2	TP53	MGMT[a]	Survival (days)
Paediatric cases										
A54	M	R. fusiform gyrus	6	Fib.	–	–	wt	wt	4.9 (3.7–12.0)	5,634[b]
A22	F	R. parietal	9	Prot.	–	–	wt	wt	2.7 (0–2.7)	6,508[b]
A35	F	R. temporal	9	Fib.	–	8q21-q22	wt	wt	4.6 (1.1–12.4)	5,654[b]
A25	M	Pons	10	Fib.	–	–	wt	wt	1.6 (0–3.5)	5,557[b]
A7	F	3rd ventricle	16	Fib.	–	–	wt	wt	3.0 (0–7.1)	460
Adult cases										
A55	M	R. frontal	21	N/A	–	–	wt	wt	4.8 (2.1–11.0)	1,311
A42	F	L. fronto-temporal	26	Fib.	–	2q, 19q	Mut	Mut	11.0 (3.3–49.9)	904
A48	M	L. tempo-parietal	26	Fib.	3p, 8q, 10p	12q	Mut	Mut	15.0 (2.6–54.8)	1,305
A50	M	R. frontal	28	Fib.	–	1p, 19q	Mut	wt	26.1 (7.6–61.3)	6,004[b]
A26	F	R. temporal	29	Fib.	7	1p, 1q, 6, 10, 13, 20	Mut	Mut	12.1 (2.7–34.1)	5,562
A30	M	R. fronto-temporal	29	Fib.	–	1p, 19q	Mut	wt	31.5 (11.3–57.7)	4,208
A9	F	L. fronto-parietal	33	N/A	2p, 7, 10p, 19q	4q, 11p, 12q, 19q	Mut	Mut	18.8 (6.2–42.7)	2,627
A6	M	L. frontal	34	Fib./Gem.	1p, 20q, 22q	6q, 11p, 13q, 22q	Mut	Mut	36.7 (11.8–69.9)	3,227
A10	M	R. temporal	36	Fib.	3p, 7q, 8q, 10p, 11q, 13q, 21q	20q, 21q	Mut	Mut	14.0 (1.5–45.4)	581
A56	F	R. frontal	38	N/A	7, 8q, 10p, 20q	3p, 15q	Mut	wt	30.5 (6.4–54.8)	42
A21	F	L. fronto-parietal	41	Fib.	7, 11q	19q	Mut	Mut	20.5 (3.1 -51.6)	3.727
A23	F	Bilat. frontal	42	Fib.	–	1p, 19q	Mut	wt	37.6 (10.8–60.6)	4,091
A12	M	R. temporal	46	N/A	3q, 4q, 7, 8p, 8q, 10p, 12p, 18p	1p, 3p, 3q, 13q	Mut	Mut	37.4 (5.1–80.4)	1,612
A40	M	R. frontal	49	Fib.	6p, 7q, 12q	3q, 6q, 13, 18q	Mut	Mut	17.6 (3.6–57.6)	6,405[b]
A49	F	R. frontal	50	Fib.	5p, 7q, 11q, 12p	6p, 13	Mut	Mut	36.4 (8.2–71.2)	4,255
A27	M	R. frontal	52	Gem.	7q, 11q	12q, 20p	Mut	Mut	44.4 (13.4–77.5)	5,776[b]

Fib fibrillary, *Prot* protoplasmic, *Gem* gemistocytic, *N/A* data not available

[a] Mean and (range) % methylation at all sites

[b] Alive at latest follow-up

defaults). The raw data for the present series have been deposited in the Gene Expression Omnibus (GEO) with accession number GSE23869.

Statistical analysis

Survival analysis was conducted using the 'survival' package in R [35]. Gene set enrichment analysis was conducted on the normalised expression data using the GSEA software available from the Broad Institute Website (http://www.broadinstitute.org/gsea) [21, 32]. Gene sets C2 (curated gene sets) and C5 (GO gene sets) from the MSig database were used, with 1,000 gene set permutations. Other statistical tests were conducted using Microsoft Excel. Unless otherwise stated, a $p < 0.05$ was considered significant.

Results

Adult diffuse astrocytoma shows a higher degree of copy number change than paediatric cases

Genome-wide copy number changes were investigated using a large-insert clone array of approximately 1 Mb resolution. Alterations affecting two or more consecutive clones were manually scored. Figure 1 shows a skyline plot of copy number changes in the adult cases ($n = 16$), and a summary is given in Table 1. Trisomy 7, gains on 7q, 8q, 10p and 11q as well as losses on 1p, 13q and 19q were all seen in greater than 20% of cases. Combined loss of 1p/19q, frequently observed in oligodendroglial tumours, was the sole change in three cases. However, none of these cases displayed features consistent with a diagnosis of

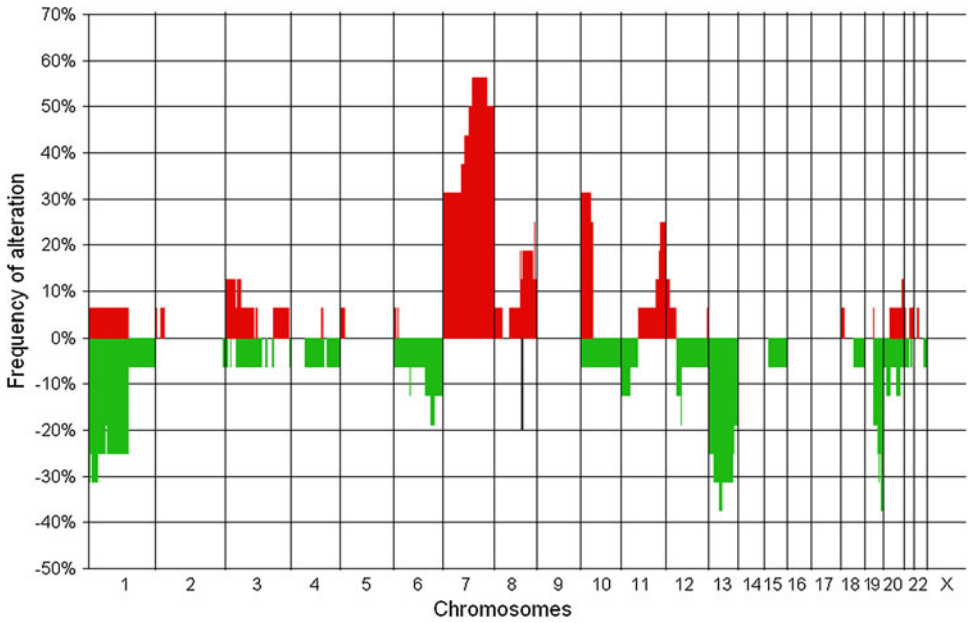

Fig. 1 A skyline plot representing copy number alterations for each array clone, ordered by genomic position, in the adult grade II diffuse astrocytomas ($n = 16$). *Green* indicates regions of gain, and *red* regions of loss. In contrast, only one small region of loss in a single case was seen in the paediatric group (*black bar*, see main text for details)

mixed oligoastrocytoma upon histological review by an experienced neuropathologist (VPC). The majority of adult cases displayed multiple aberrations. Four out of five paediatric tumours, however, displayed entirely normal copy number at this resolution (Table 1). Only one paediatric tumour, A35, displayed a small region of loss encompassing clones RP11-3J21 and RP11-388K12 at 8q21-q22 (maximally, Chr8:92484240-95267614). The known coding genes in this region are *RUNX1T1*, *C8orf83*, *FAM92A1*, *RBM12B*, *TMEM67*, *PDP1*, *CDH17* and *GEM*. A recent report described alterations affecting the *MYB* gene in a subset of AII [34]. No copy number changes at this locus were observed in any sample in our series, despite the array containing a clone covering the whole of the *MYB* gene (RP1-32B1). Further, no alterations at the *BRAF* locus on 7q34, common in pilocytic astrocytoma, were seen in any sample [14].

Mutations of *IDH1/2* and *TP53* are common in adult but rare in paediatric AII

All samples were assessed for mutations in exon 4 of both *IDH1* and *IDH2*, encompassing the hotspot codons R132 and R172, respectively. 13 of the 16 adult cases showed *IDH1* mutation, and a further two cases had a mutation in *IDH2*. Thus, 94% of the adult cases showed an *IDH1/2* alteration. In contrast, no *IDH* mutation was observed in any of the childhood tumours.

The whole coding sequence and flanking intronic sequences of *TP53* were also screened for mutations. 11 of the 16 adult cases (69%) displayed a mutation in *TP53*. In contrast, no mutations were seen in any of the paediatric cases. *TP53* mutation was mutually exclusive to combined 1p/19q loss, meaning 14/16 adult cases possessed an *IDH1/2* mutation and either 1p/19q loss or *TP53* mutation. One further case had an *IDH1* mutation alone. Of note, the only adult case lacking any of these alterations was also the youngest in the adult series, at 21 years of age. These data are summarised in Table 1.

To further support these data, a meta-analysis of the literature was conducted to expand the number of both adult and paediatric cases of AII. This revealed a combined frequency of *TP53* mutation of 6/87 (7%) of paediatric cases, and 41/73 (56%) of adult AII, $p < 1 \times 10^{-11}$, two-tailed Chi-squared test. In the case of *IDH1/2*, the frequency was 5/32 (16%) of paediatric AII, and 316/389 (81%) of adult AII, $p < 1 \times 10^{-16}$, two-tailed Chi-squared test. Data and references are summarised in Supplementary Table 2.

Adult AII carry a less favourable prognosis than their paediatric counterparts

At latest follow-up (October 2009), 80% of the paediatric cases (4/5) were still alive, when compared with only 19% (3/16) of the adult group. Median follow-up times for survivors were 15.5 (paediatric) and 16.4 years (adult). Median survival in the adult group was 9.5 years, while it was not reached in the paediatric cases. 15-year overall survival rates were 80 and 25% for childhood and adult cases, respectively. Kaplan–Meier curves for the two groups are shown in Fig. 2. A log-rank test revealed only a marginal p value of 0.056 for this difference, due to the low number of cases. However, this association of age and prognosis is further supported by data collected by the Central Brain Tumor Registry of the United States between 1995 and 2006

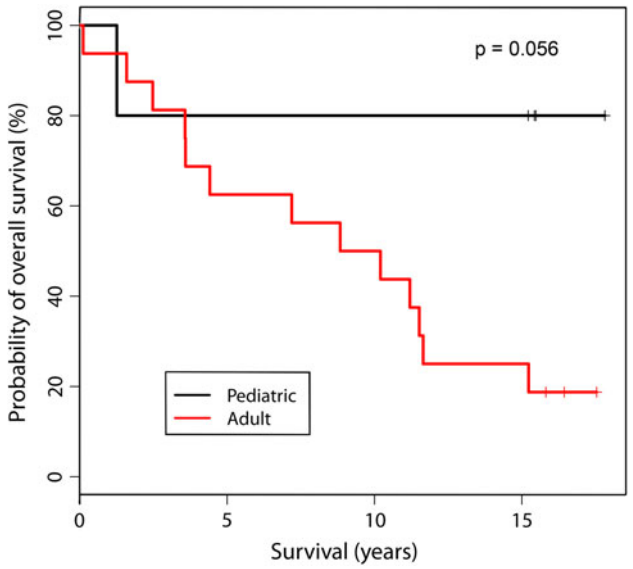

Fig. 2 Kaplan–Meier survival curves for the adult (*red line* $n = 16$) and paediatric (*black line* $n = 5$) diffuse astrocytoma (WHO grade II). *Vertical bars* indicate follow-up censored at this time point. A log-rank test was used to calculate the significance of this survival difference

[4]. These data show that for protoplasmic and fibrillary astrocytoma grade II, 10-year survival rates were 81.2% for the 0–19 years age group ($n = 100$), while 10-year survival in 20–44 year olds was only 39.2% ($n = 221$).

MGMT is methylated only in adult AII

Methylation of 16 CpG dinucleotides from position +106 to +213 relative to the transcription start site was quantitatively assessed by pyrosequencing. This region covers the CpGs interrogated by the commonly used MSP assay (+118 to +137, and +174 to +195) [6]. All five paediatric cases were unmethylated (mean methylation <5% in all cases, maximum methylation at any CpG site ranging from 3 to 12%). In contrast, the mean CpG methylation in this region was more than 20% in 9/16 adult cases, and 15/16 had at least one CpG site with more than 30% methylation. Quantitative RT-PCR on a subset of samples (see "Materials and methods") also showed a significant reduction in *MGMT* expression in adult when compared with paediatric cases ($p < 0.05$, two-tailed Student's *t* test, data not shown).

Global expression profiling clearly distinguishes adult and childhood AII

To get an impression of the global transcriptional profile of grade II diffuse astrocytomas, 10 cases were analysed using an Illumina HumanHT-12 expression chip. This included all five paediatric samples, and adult cases selected on the basis of high quality RNA: A9, A26, A30, A48 and A55.

Principle component analysis revealed a very clear distinction between the expression patterns of the adult and paediatric cases, as shown in Fig. 3a. The limma package was then used to determine genes differentially expressed between the adult and paediatric groups with a $p < 0.05$ after empirical Bayes analysis and multiple testing correction. The most striking pattern to emerge from this list was a large number of genes with a demonstrated or putative role in neural stem cell self-maintenance/self-renewal or CNS development, as discussed further below. A complete list of 56 genes with an expression change of twofold or greater and $p < 0.05$ is given in Supplementary Table 3a. To further assess the pathways affected by differential expression in the two age groups, we applied the method of gene set enrichment analysis (GSEA). The full lists of the top 100 marker genes for each age group are given in Supplementary Table 3b, showing a large degree of overlap with the differential genes by Bayesian analysis, as expected. The expression of the top 50 marker genes for each group is illustrated in Fig. 3b. At a cut off of $p < 0.01$ and FDR < 0.25, 33 gene sets were enriched in the adult subgroup, and 414 in the paediatric tumours. These groups are listed in Supplementary Table 3c. Intriguingly, the top gene sets enriched in the adult population included 'Negative Regulation of Cell Differentiation', and also several lists linked to cell cycle progression and DNA replication, as discussed below. In the paediatric genes, one of the top enriched sets was 'Lei Myb Targets', a set of genes showing differential expression as a result of Myb transgene activation in cell lines [17]. This is of interest given a recent report on the role of *MYB* in paediatric AII, also discussed below [34].

Quantitative RT-PCR analysis was used to validate some of these alterations in 17 cases for which cDNA was available. Figure 3c shows the difference in expression levels by age group of *DLL3*, *HES5*, *BMP2, TIMP1* and *BAMBI,* chosen for their significantly differential expression by Bayesian analysis and position in the top 100 of the GSEA marker gene list, as well as a putative role in CNS development as discussed below. For *HES5, TIMP1* and *BAMBI* a significant difference was confirmed ($p < 0.05$, Student's *t* test). For *DLL3* and *BMP2*, the trend towards lower expression in paediatric cases was confirmed, although these differences did not reach statistical significance by this method ($p < 0.1$ and $p < 0.15$, respectively; Student's *t* test).

Discussion

The data presented here indicate fundamental differences between adult and childhood cases of diffuse astrocytoma

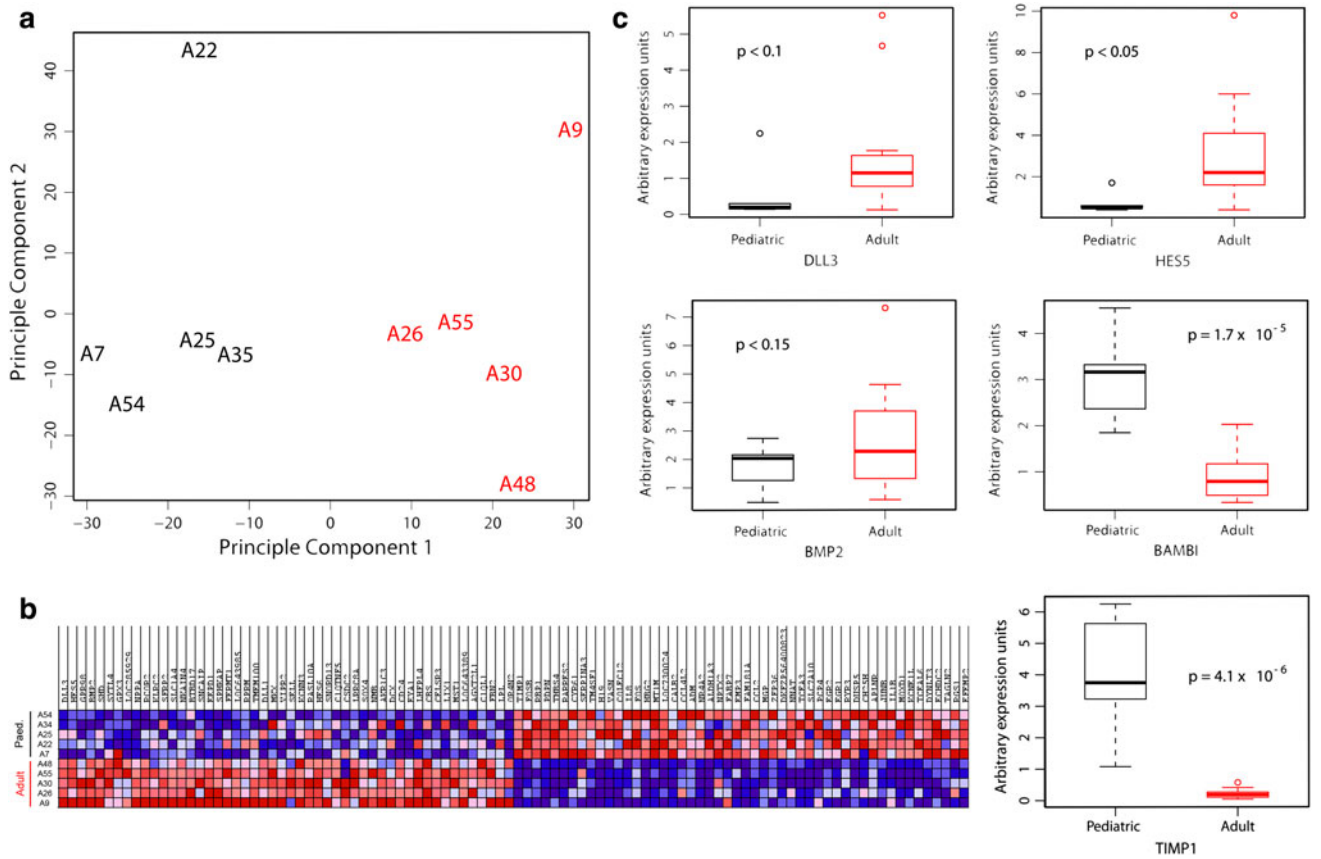

Fig. 3 **a** Principal component analysis on global gene expression profiles (17,106 probes, see main text for details) of grade II diffuse astrocytomas clearly distinguishes the adult (*red*) and paediatric (*black*) groups ($n = 5$ per group). **b** Heatmap displaying the expression of the top 50 marker genes for the adult and paediatric groups as determined by GSEA. **c** Boxplots showing quantitative PCR analyses of *DLL3, HES5, BMP2, TIMP1 and BAMBI* for paediatric (*black*, $n = 5$) and adult (*red*, $n = 12$) cases. *p* values were calculated using Student's *t* test

(WHO grade II) in several key aspects of their constitution, as revealed by describing an integrated analysis of global copy number changes, target gene mutation and methylation status, clinical follow-up and gene expression profiles. This is in keeping with a small number of reports on the pathogenesis of these tumours that have also suggested age-related genetic differences in certain aspects of their biology [7, 22, 36]. A similar distinction has also been suggested in high-grade gliomas [27].

Genetically, there are a number of marked distinctions. The younger patients displayed broadly normal genetic complements in terms of DNA copy number, while adult patients typically showed multiple alterations. The paediatric cases also lacked mutations in the classic AII target genes *IDH1/2* or *TP53*, which were present in 94 and 69%, respectively of the adult cases in our series. This is also evidenced by further examples in the literature, as described above, and suggests a different genetic aetiology between the majority of adult and childhood cases. The use of *IDH* mutation status as an indicator of better prognosis, as described elsewhere, is clearly not supported in the paediatric population [10].

The relationship between *IDH1/2* mutation and patient age is intriguing in light of previous reports, since several studies actually describe a younger average age of onset for adults with tumours carrying an *IDH* gene mutation [2, 8, 31, 37, 38]. A caveat to this, however, is that mutations in the youngest subset of patients are extremely uncommon. For example, the youngest patient with an *IDH* mutation that we reported in a broader series of 364 gliomas was 17 years of age (while 24 patients in the series were younger than 17) [12]. In two further studies, 0/23 children <14 years old and only 4/32 case <18 years old showed a mutation [8, 28]. Although *IDH* mutation is therefore likely to be an early event in tumourigenesis, it is very rare in teenagers and children.

The adult and paediatric cases examined here also demonstrated a dramatic difference in their outcome, with 80% of paediatric cases surviving after 15 years when compared with a median survival in the adult group of

<10 years and a 15-year survival rate of only 25%. There is no evidence that this difference can be ascribed to therapeutic strategies, because most of the adult patients received additional adjuvant therapy (12/16 received radio- and/or chemotherapy) and might, therefore, be expected to fare better than the childhood cases (treated with surgery alone). The distribution of tumour locations is also similar across the series. Thus, it seems likely that these survival differences (further supported by a larger CBTRUS series, as noted above) are reflective of true biological distinctions between the tumours. A recent reported described a subpopulation of grade II gliomas lacking IDH mutation that displayed a worse prognosis than IDH mutant cases [20]. However, this study examined only adult patients. Because 94% of our adult cases possessed an IDH mutation, it was not possible to draw a similar comparison. Indeed, in our combined series, lack of IDH mutation actually indicated a better prognosis, because lack of this mutation was associated with the paediatric cases.

The expression profiles of the two tumour groups provide an interesting suggestion as to one potential reason for this dichotomous behaviour. Principle component analysis on global expression profiles clearly distinguished between adult and paediatric cases. In comparison with their childhood counterparts, adult tumours were characterised by an expression pattern which may be indicative of a more stem-like, less differentiated cell of origin with a greater replicative potential and ability to progress. Some of the gene sets enriched in adults by GSEA included 'Mitosis', 'DNA Replication Reactome', 'Cell Cycle Process' and 'Negative Regulation of Cell Differentiation'. Genes showing significantly increased expression in adult tumours by Bayesian analysis included several members related to the Notch signalling pathway, responsible for neural stem cell (NSC) self-renewal [13]. For example, *hairy and enhancer of split 5* (*HES5*), which represses the transcription of proneural genes [15], was upregulated fourfold in our expression analysis. *DLL3*, a Notch ligand again putatively involved in NSC maintenance [39], was the most highly upregulated gene in the adult cases. In addition, upregulated in adult tumours was *bone morphogenetic protein 2* (*BMP2*), which can induce *HES5* expression to silence neuronal gene expression and promote a more astroglial phenotype [23]. The *BMP and activin membrane-bound inhibitor* (*BAMBI*) gene, encoding an antagonistic pseudoreceptor for BMP2, was concordantly downregulated in the adult tumours. A further gene regulating this putative aberrant Notch/NSC axis is the basic helix-loop-helix transcription factor, *BHLHB2*. This gene, loss of which has been shown to increase cellular proliferation and decrease differentiation by elevating Notch signalling, was downregulated in the adult tumours [33]. B-*cell translocation gene 2* (*BTG2*), which normally induces ATOH1 (resulting in neuronal differentiation) and down-regulates cyclin D1 [3], was also underexpressed in adult cases.

Interestingly, one of the highest ranking enriched gene sets in the paediatric group was the 'Lei Myb Targets' set—a list of genes altered in expression in response to ectopic Myb expression in cell lines [17]. This fits well with a recent report of alterations affecting *MYB* both in terms of copy number alterations and increased protein expression in paediatric low-grade gliomas [34]. The authors therefore believe that the contribution of *MYB* and associated pathways to the biology of paediatric AII is worthy of further investigation.

Taken together, these expression changes suggest a difference in differentiation state and proliferative potential between the two age groups, which may reflect a divergence in the progenitor pool from which they derive, and which likely plays a role in determining the distinctive behaviour of these two entities. That this is a true tumour-specific difference, rather than merely representing normal differences in gene expression with age, is supported by the fact that analysis of over 20 pilocytic astrocytomas of varying ages on the same platform revealed no similar distinction by PCA. Both adult and paediatric cases also clustered independently from normal brain samples (our unpublished data).

Diffuse astrocytoma (WHO grade II) is often included under the umbrella term 'low-grade astrocytoma'. In the paediatric population, the majority of patients seem to have good long-term survival prospects, in keeping with WHO grade I tumours. Notably, however, none of the childhood AII in our series possessed a *RAF* gene fusion or mutation, or *KRAS* mutation (data not shown)—a common finding in pilocytic astrocytoma (grade I, PA) [14]. The adult AIIs presented here are also clearly different in a number of aspects from PAs and such terms as 'low-grade astrocytoma' are therefore misleading. Furthermore, distinct genetic, survival and expression profiles suggest that histologically similar AIIs occurring in children and adults may in fact be two different entities deriving from independent progenitor cell pools and/or divergent tumourigenic mechanisms. Further elucidation of this distinction in a larger sample cohort will be vital in informing future investigations into the development of these tumours, and potential therapeutic strategies to combat them.

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Conflict of interest The authors declare that they have no conflict of interest.

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