Pediatric High-Grade Astrocytomas Show Chromosomal Imbalances Distinct from Adult Cases

Christian H. Rickert,^{*¶} Ronald Sträter,[†]
Peter Kaatsch,[‡] Hansdetlef Wassmann,[§]
Heribert Jürgens,[†]
Barbara Dockhorn-Dworniczak,[¶] and
Werner Paulus^{*}

From the Institute of Neuropathology,^{*} the Department of Pediatric Oncology,[†] the Department of Neurosurgery,[§] and the Gerhard-Domagk-Institute of Pathology,[¶] Westfälische Wilhelms-Universität Münster, Münster; and the German Childhood Cancer Registry,[‡] Institute for Medical Statistics and Documentation, Johannes Gutenberg-Universität, Mainz, Germany

We studied 23 pediatric high-grade astrocytomas by comparative genomic hybridization. Chromosomal imbalances were found in 10 of 10 anaplastic astrocytomas and 11 of 13 glioblastomas and consisted of +1q (43%), +3q (26%), +1p, +2q, +5q (22%), -22q (34%), -6q, -10q (30%), -9q, -11q, -13q, -16q, and -17p (22%). Anaplastic astrocytomas frequently showed +5q (40%), +1q (30%), -22q (50%), -6q, -9q (40%), and -12q (30%); glioblastomas +1q (54%), +3q (38%), +2q, +17q (23%), -6q, -8q, -10q, -13q, and -17p (31%). Minimal common regions mapped to +1q21-41, +3q27-qter, +2q31-32, +5q14-22, -22q12-qter, -10q23-25, -6q25-qter, -9q34.2, -11q14-22, -16q22-qter, and -17p. High-level gains were located on 1q (7 cases), 2q, 7q (4 cases), 3q (3 cases), 9, 17q (2 cases), 4q, 8q, 18, and 20q (1 case). A significantly shorter survival was found for anaplastic astrocytomas showing +1q ($P < 0.05$), MIB-1 proliferation index $>25\%$ ($P < 0.001$) and glioblastomas ($P < 0.05$). Compared with adult cases, +1p, +2q, and +21q as well as -6q, -11q, and -16q were more frequent in pediatric malignant astrocytomas. Among the latter +5q, -6q, -9q, -12q, and -22q were characteristic for pediatric anaplastic astrocytomas and +1q, +3q, +16p, -8q, and -17p for pediatric glioblastomas. Our results show that chromosomal aberrations differ between pediatric anaplastic astrocytomas and glioblastomas as well as between pediatric and adult high-grade astrocytomas, supporting the notion of a different genetic pathway. Furthermore, gains of chromosomal material on 1q might be correlated with a worse prognosis in pediatric anaplastic astrocytomas. (*Am J Pathol* 2001, 158:1525-1532)

Astrocytomas are the most common pediatric brain tumors and account for 40.9% of cerebral and 7.7% of all childhood cancers.¹ High-grade astrocytomas, ie, anaplastic astrocytomas and glioblastomas, however, are rarer compared with their adult counterparts and make up 6.6% of pediatric brain tumors.¹ Relatively few studies have been performed on their molecular properties, and different genetic pathways seem to be involved in the evolution of pediatric and adult high-grade astrocytomas.

Comparative genomic hybridization (CGH) is able to identify imbalances of the entire genome in terms of DNA copy number changes. Its main advantage is that it bypasses the need for laborious cell culture to harvest metaphase spreads, and that it can be applied to archival material. Although it has previously been used in a few studies on adult high-grade astrocytomas, no CGH study has hitherto been undertaken on pediatric high-grade astrocytomas. To screen for DNA copy number changes that may be involved in tumorigenesis of pediatric malignant astrocytomas, we applied CGH on anaplastic astrocytomas and glioblastomas from 23 patients under 18 years of age.

Materials and Methods

Patients and Tumors

Formalin-fixed and paraffin-embedded biopsy specimens of 23 primary pediatric high-grade astrocytomas were investigated (Table 1). These consisted of 10 anaplastic astrocytomas World Health Organization grade III (three males, seven females; mean age, 7.1 years; range, 8 months to 17 years) and 13 glioblastomas World Health Organization grade IV [six males, seven females (including two sisters; cases 12 and 13); mean age, 8.9 years; range, 1 to 16 years]. Of these, 20 tumors were located supratentorially (9 anaplastic astrocytomas, 11 glioblastomas), two in the cerebellum (one anaplastic astrocytoma, one glioblastoma), and one in the brain stem (one glioblastoma). The pediatric age group was defined as patients under 18 years of age at the time of operation. All patients underwent surgery or stereotactic biopsy. A combination of postoperative radiation therapy and chemotherapy was applied to eight patients, whereas 10

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Address reprint requests to Christian H. Rickert, M.D., University of Münster, Institute of Neuropathology, Domagkstr. 19, D-48129, Münster, Germany. E-mail: rickchr@uni-muenster.de.

Table 1. Clinical Data of 23 Biopsy Specimens from Pediatric Anaplastic Astrocytomas and Glioblastomas

Case no.	Age, years	Sex	MIB	Location	Therapy	Clinical course	Survival, months
Anaplastic astrocytomas (WHO III)							
1	0.7	M	13.9	Parietal	CT	Stable disease	>27
2	8	F	3.0	Parietal	RT/CT	Free of disease	>48
3	16	F	5.9	Temporal	RT/CT	Progress	20
4	6	F	20.9	Hypothalamus	RT/CT	Remission	>30
5	17	F	7.8	Parietal	RT	Remission	>38
6	5	F	37.6	Parietal	—	Progress	3
7	4	M	38.5	Parietal	RT/CT	Progress	4
8	0.7	F	12.2	Temporal	RT/CT	Remission	>102
9	12	M	3.6	Cerebellum	RT	Remission	>77
10	2	F	16.8	Temporal	—	Progress	3
Glioblastomas (WHO IV)							
11	15	M	11.7	Fronto-parietal	RT	Free of disease	>23
12	5	F	50.8	Temporal	RT	Progress	1
13	7	F	49.7	Parietal	RT	Progress	6
14	4	M	41.9	Paraventricular	RT/CT	Progress	11
15	11	M	24.2	Brain stem	RT/CT	Progress	31
16	4	F	36.8	Parietal	RT	Progress	15
17	6	F	39.9	Parieto-occipital	RT/CT	Progress	8
18	12	F	12.6	Frontal	RT/CT	Progress	12
19	15	M	33.4	Thalamus	RT	Progress	9
20	14	F	51.1	Occipital	RT	Progress	16
21	5	M	26.1	Parietal	RT	Progress	11
22*	16	M	22.2	Temporal	RT	Progress	8
23	1	F	12.8	Cerebellum	CT	Remission	>8

M; male, F; female, MIB-1; proliferation index in percent; RT; radiotherapy; CT; chemotherapy.

*Giant cell glioblastoma.

patients underwent radiation therapy and three patients underwent chemotherapy alone; two children did not receive any adjuvant therapy (Table 1). Clinical follow-up data were available for all patients.

Histological criteria for classifying a tumor as anaplastic astrocytoma or glioblastoma were those of the World Health Organization classification.² Only tumor samples that had been shown histologically to contain >70% of tumor cells were included. Routine hematoxylin and eosin staining and immunohistochemistry using an avidin-biotin complex technique and monoclonal antibodies against glial fibrillary acid protein as well as the proliferation antigen Ki-67 (clone MIB-1) were performed. Statistical analysis was performed using Kaplan-Meier estimates of survival probabilities and the log-rank test.

CGH Analysis

DNA was isolated by phenol-chloroform extraction according to standard protocols. With minor modifications, CGH analysis was performed as described previously.³ Briefly, tumor DNA was labeled with biotin-16-dUTP (Boehringer Mannheim, Mannheim, Germany) and reference DNA from a healthy male donor with digoxigenin-11-dUTP (Boehringer Mannheim) in a standard nick translation reaction. The DNase concentration in the labeling reaction was adjusted to reveal an average fragment size of 200 to 500 bp. The labeled DNA fragments were purified from remaining nucleotides by column chromatography.

For CGH, 500 ng of tumor DNA, 300 ng of reference DNA, and 30 μ g of human Cot1 DNA (Gibco, Karlsruhe,

Germany) were co-precipitated and redissolved in 10 μ l of hybridization buffer. Denaturation of DNA (75°C for 5 minutes) was followed by a pre-annealing time of 45 minutes at 37°C. Target metaphase spreads (46,XY), which had been prepared following standard procedures, were denatured separately in 70% formamide/2 $\times$ standard saline citrate for 2 minutes at 72°C. Hybridization was allowed to proceed for 3 to 4 days. Posthybridization washes were performed to a stringency of 50% formamide/2 $\times$ standard saline citrate at 45°C and 0.1 $\times$ standard saline citrate at 60°C. Biotinylated and digoxigenated sequences were detected simultaneously, using avidin-fluorescein isothiocyanate (1:200; Boehringer Mannheim) and anti-digoxigenin-rhodamine (1:40; Boehringer Mannheim). The slides were counterstained with 4,6-diamidino-2-phenylindole (DAPI) and mounted in an anti-fade solution (Vectashield; Vector Laboratories, Burlingame, CA).

Microscopy and Digital Image Analysis

Separate digitized gray level images of DAPI, fluorescein isothiocyanate, and rhodamine fluorescence were taken with a charge-couple device camera connected to a Leica DMRBE microscope (Leica, Wetzlar, Germany). The image processing was performed by use of Applied Imaging Software (Applied Imaging, Sunderland, UK). Average green-red ratios were calculated for each chromosome in at least 10 metaphases.

Chromosomal regions with CGH ratio profiles surpassing the 50% CGH thresholds (upper threshold, 1.25; lower threshold, 0.75) were defined as loci with copy

Table 2. Gains and Losses in DNA Copy Number by CGH

Case	Gains (respective top lines) and losses (respective bottom lines)
Anaplastic astrocytomas (WHO III)	
1	- 9q34.2, 10q23-25
2	- 9
3	1, 2, 4q , 7, 8q, 15q, 16, 17q22-qter, 20q , 21 3q, 5q, 6q25-qter, 8p, 10q23-25, 12q21.2-qter, 13q, 18, 22q12-qter
4	2q31-32, 3q25-qter, 4, 5p, 5q11.2-23.2, 9 , 20, 21q 1p, 6, 7q, 8, 10q, 11q, 12q, 16, 22q
5	- 9
6	- 6q24-qter, 11q14-22, 22
7	1, 5q11.2-23.2, 7 , 9 3p22-24, 6q, 12, 17p, 21q
8	5q11.2-22, 6q12-16, 13q22 16, 17q24-qter, 22q12-qter
9	5q14-23, 6q12-22, 12q21 1, 3, 9q22-qter, 22q12-qter
10	1q
Glioblastomas (WHO IV)	
11	1p34.2-pter, 16
12	- -
13	1q21-41 -
14	8q 6q14, 6q22-qter, 9q, 13q12-21, 17p
15	1q , 2 , 3q , 8q , 17q23-qter 14q, 16q, 20p
16	1p31.2-34.2, 1q , 2(2q23-32), 3q26.2-qter, 7 , 17q , 18 4q28.2-qter, 6q16.2-qter, 8q, 10, 11q, 16, 17p
17	- -
18	3q27-qter 4q28.2-qter, 5q13-15, 9p, 10q, 13q, 22q
19	1q, 3q27-qter, 12q24.3, 17q 7q22-31, 16q22-qter, 18p
20	1q -
21	1 (1q42-qter), 2q (2q23-34), 3q26.1-qter, 7 (7q), 9q13-qter, 16, 20, 21q 6, 8q, 10q, 11, 12q23-qter, 13q31-qter, 15q, 17p, 18q12-qter, 22q12-qter
22	1q, 4, 5p, 5q11.2-21, 21 2q14.1-qter, 6q14-qter, 8q, 10q, 11, 13q, 14q, 17p, 22q
23	16p -

Bold type indicates high level gains.

number gains or losses. Based on experiments with normal control DNA, these thresholds have been shown to eliminate false-positive results. These values have been used in several studies comparing CGH data with results obtained by other cytogenetic methods and have proven to provide robust criteria for the diagnosis of chromosomal gains and losses. Overrepresentations were diagnosed as high-level gains or amplifications when the fluorescence intensity levels exceeded 1.5 or when the fluorescein isothiocyanate fluorescence showed strong focal signals. For the assignment of these high-level amplifications to chromosomal bands, the signal intensities were compared to the DAPI banding on individual chromosomes. As tumor specimens and normal DNA were not sex matched, X and Y chromosomes were excluded. Also excluded were centromeric and satellite regions of

the acrocentric chromosomes and chromosome 19 because of the abundance of highly repetitive DNA sequences as well as the frequent occurrence of false-positive CGH results as shown by interphase fluorescence *in situ* hybridization using suitable DNA probes.

Results

CGH revealed DNA copy number changes in 10 of 10 anaplastic astrocytomas and 11 of 13 glioblastomas; six patients showed only one chromosomal imbalance each (Table 2). Every chromosome was shown to carry imbalances (Figure 1); most imbalances were found on chromosomes 1 (14 cases), 6 (11 cases), and 17 (10 cases),

Figure 1. CGH ideogram summarizing gains and losses of DNA sequences in pediatric anaplastic astrocytomas (gray) and glioblastomas (black). Gains are shown as **bars** on the **right** side of the chromosome ideogram and losses on the **left**. High-level amplifications are marked as **dotted lines**. Each vertical represents the affected chromosomal region seen in a single tumor specimen.

the fewest on chromosomes 14 and 15 (2 each). The most DNA copy number changes were detected in an anaplastic astrocytoma (19 changes), the fewest in two glioblastomas (none). Anaplastic astrocytomas showed an average of 6.6 chromosomal changes per tumor (range, 1 to 19; 2.9 gains *versus* 3.7 losses), which were more common than in glioblastomas with an average of 6.1 changes per tumor (not significant; range, 0 to 18; 2.8 gains *versus* 3.3 losses) (Table 1, Figure 1). Losses were more common than gains in both entities (not significant).

The most common DNA copy number changes overall were gains on 1q (43%), 3q (26%), 1p, 2q, and 5q (22%) as well as losses on chromosomes 22q (34%), 6q, 10q (30%), 9q, 11q, 13q, 16q, and 17p (22%). In anaplastic astrocytomas, the most frequent imbalances were gains on 5q (40%) and 1q (30%) as well as losses on chromosomes 22q (50%), 6q, 9q (40%), and 12q (30%). In glioblastomas, the most common DNA copy number changes were gains on 1q (54%) and 3q (38%), 2q, and 17q (23%) as well as losses on chromosomes 6q, 8q, 10q, 13q, and 17p (31%) (Table 1, Figure 1).

Minimal common regions found in at least five tumors consisted of gains of 1q21-41 (10 cases), 3q27-qter (6 cases), 2q31-32, and 5q14-22 (5 cases) as well as losses of 22q12-qter (8 cases), 10q23-25, 6q25-qter (7 cases),

9q34.2, 10q, 11q14-22, 16q22-qter, and 17p (5 cases). High-level gains affecting whole chromosomes, as indicative of trisomy, were found on chromosomes 7 (3 cases), 1, 2, and 9 (2 cases), as well as on 18 (1 case). High-level gains affecting parts of a chromosome or chromosome arms were found on chromosomes 1q (7 cases), 2q, 7q (4 cases), 3q (3 cases), 17q (2 cases) as well as 4q, 8q, and 20q (1 case) (Figure 1). Between one and five high-level gains were found in four of the anaplastic astrocytomas (cases 3, 4, 7, and 10), and between one and six in five of the glioblastomas (cases 15, 16, 19, 20, and 21) (Table 1).

The mean MIB-1 proliferation indices were highly significantly different between anaplastic astrocytomas and glioblastomas and were 17.0% (range, 3.0 to 38.5%; SD, 12.2%) for anaplastic astrocytomas and 31.8% (range, 11.7 to 51.1%; SD, 14.6%) for glioblastomas ($P < 0.01$). All children could be followed-up, and the median overall survival was 15.0 months (SD, 3.8 months). At the time of reporting, 6 of 10 children with anaplastic astrocytomas were still alive (no median overall survival calculable because $>50\%$ of patients were still alive at time of reporting; mean overall survival, 64.2 months; SD, 14.7 months) whereas only 2 of 13 children with glioblastomas were still alive (median overall survival, 11.0 months; SD,

1.7 months; mean overall survival, 13.5 months; SD, 2.7 months); the difference in survival was statistically significant ($P < 0.05$). The respective 1-, 2-, and 3-year survival rates were 55, 33, and 21% for all malignant astrocytomas; 70, 60, and 50% for anaplastic astrocytomas; and 46, 9, and 0% for glioblastomas. A highly significantly shorter survival was found for all high-grade astrocytomas showing a MIB-1 proliferation index of $>25\%$ (median overall survival, 8.0 months; SD, 2.4 months; mean survival, 8.4 months; SD, 1.6 months) compared with tumors with a MIB-1 proliferation index of $<25\%$ (no median overall survival was calculable because $>50\%$ of patients were still alive at time of reporting; mean survival, 63.0 months; SD, 13.4 months; $P < 0.001$). Although the number of aberrations overall as well as of gains and losses on their own bore no significance on survival, a significantly shorter survival was found among pediatric anaplastic astrocytomas showing gains of 1q (median overall survival, 9.0 months; SD, 2.4 months; mean survival, 12.3 months; SD, 2.7 months) compared with cases without this imbalance (no median overall survival calculable because $>50\%$ of patients were still alive at time of reporting; mean survival, 87.9 months; SD, 13.3 months; $P < 0.05$) (Figure 2a) although no such correlation could be found for pediatric glioblastomas in which cases with +1q as well as those without +1q showed a median overall survival of 11.0 months ($P = 1.00$; Figure 2b). No correlation could be found between prognosis and age, gender, or year of diagnosis.

Discussion

Although malignant astrocytomas are fairly uncommon brain tumors in children, they are of great clinical interest regarding their potential therapeutic approach and improvement of prognosis that has led to the establishment of several multicenter treatment studies. However, little is known about their molecular biology and genetics with somewhat conflicting results. In most cytogenetic investigations, the main point of contention, particularly in view of the sometimes surprisingly long survival of children suffering from these tumors and the fact that astrocytic tumors with similar degrees of histological anaplasia often show different biological behavior, focused on whether pediatric and adult high-grade astrocytomas follow similar⁴⁻⁶ or different⁷⁻¹³ genetic pathways, and whether they show consistent or varying chromosomal changes. To date, no CGH study on primary pediatric anaplastic astrocytomas and glioblastomas has been reported.

Pediatric High-Grade Astrocytomas

Although a normal karyotype was found in 14 out of 22 pediatric anaplastic astrocytomas,¹⁴ anaplastic astrocytomas all showed DNA copy number changes in our series. These were mainly characterized by gains of 5q and losses of 22q and 9q, whereas glioblastomas predominantly showed gains of 1q and 3q as well as losses of 6q, 10q, 13q, and 17p (Figures 1 and 3). Some of these imbalances have previously been reported in

Figure 2. Kaplan-Meier survival estimates showing significantly lower survival probabilities for children with anaplastic astrocytomas showing gains of 1q compared with cases without this change (A; $P < 0.05$), whereas no prognostic significance of +1q could be found in pediatric glioblastomas (B).

pediatric malignant astrocytomas, namely losses of chromosomes 13,⁹ 17p,^{5,6,9,15} and 22q.^{5,6,14} Furthermore, losses of heterozygosity at 17p13.1, 9p21, and 10q23-25 were found in 50, 83, and 78% of high-grade pediatric astrocytomas, respectively.¹³ TP53 tumor suppressor gene mutations (mapping to 17p13.1) have been found in 38% of pediatric high-grade astrocytomas,¹³ 25% of glioblastomas,^{12,16} and 11% of anaplastic astrocytomas¹⁶ as well as 71% of brain stem glioblastomas,¹⁷ underscoring the proposed preferential inactivation of the p53 tumor suppressor pathway in $>95\%$ of pediatric astrocytomas.¹⁸ On the other hand, mutations of the PTEN gene (mapping to 10q23-25) are rare in pediatric cases in which they are only found in $\sim 8\%$ of tumors;^{11,13} in our series chromosomal material of 10q was lost in 31% of tumors. Furthermore, no MDM2 (12q13-21)¹⁵ or epidermal growth factor receptor (EGFR, mapping to 7q21-32) gene amplifications were detected in several investigations,^{12,13,17-19} which is corroborated by the few CGH changes found by us at the aforementioned sites.

Figure 3. Comparison of CGH data of pediatric (gray) and adult (black) anaplastic astrocytomas (**top**) as well as pediatric (gray) and adult (black) glioblastomas (**bottom**), charted against each affected chromosomal arm. Relative frequency of chromosomal imbalances in percent; gains charted above, losses below the respective midlines. CGH results of primary adult high-grade astrocytomas gained from 22 anaplastic astrocytomas²¹⁻²⁴ and 184 glioblastomas.²¹⁻²⁹

A review of cytogenetic data from ~200 pediatric glioblastomas demonstrated gains of chromosome 7 to be the most common imbalance encountered in 45% of tumors, followed by losses of 10, 9p, and 22q in 35, 20, and 17% of cases, respectively; very similar cytogenetic changes were reported for 43 pediatric anaplastic astrocytomas.¹⁴ However, these aberrations as well as losses of other implicated chromosome regions like 1q¹⁷ and 10p^{5,6} played little or no role among our cases, except for losses of 22q in anaplastic astrocytomas and 10q in glioblastomas (Figure 1). In contrast, our cases showed frequent and prognostically relevant gains of 1q, the most commonly amplified chromosomal region overall and associated with a poorer outcome, and one that has previously been implicated in pediatric low-grade but not hitherto in high-grade astrocytomas,²⁰ and losses of 9q rather than 9p (Figure 1).

CGH in Pediatric and Adult High-Grade Astrocytomas

CGH results on primary adult high-grade astrocytomas have been presented in nine studies consisting of 22 anaplastic astrocytomas²¹⁻²⁴ and 184 glioblastomas.²¹⁻²⁹ Loss

of genetic material of 10q was the most common chromosomal change overall and similarly frequent in the pediatric primary anaplastic astrocytomas and glioblastomas of this series (30 and 31%, respectively) and adults (32 and 59%) alike (Figure 3). Multiple tumor suppressor genes on the long arm of chromosome 10 have been implicated in the development of astrocytic gliomas:³⁰ among glioblastoma mutations, deletions, and loss of heterozygosity on 10q have been found more frequently in primary compared with secondary glioblastomas and have predominantly been associated with the PTEN gene.^{31,32} Gains of chromosome 7, containing the EGFR gene that is amplified in >30% of adult malignant astrocytomas,³³ and losses of 9p, although, were much more frequently found in adult malignant astrocytomas (32 to 58%) compared with pediatric cases (8 to 20%), whereas losses of 10p exclusively occurred among adult anaplastic astrocytomas and glioblastomas (27 and 51%) but not in any of our pediatric cases. In contrast, there was a marked prevalence for pediatric anaplastic astrocytomas and glioblastomas to show gains of chromosomes 1p (20 and 23% versus 5 and 9%), 2q (20 and 23% versus 9 and 3%), and 21q (20 and 16% versus 0 and 3%) as well as losses of 6q (40 and 31% versus 9 and 18%), 11q (20 and 23% versus 5 and 6%), and 16q (20 and 23% versus 5 and 5%). However, a recent microsatellite study on astrocytic tumors found losses affecting chromosome 6q in 38% of anaplastic astrocytomas and 37% of glioblastomas.³⁴ Chromosomal imbalances that seemed to be more characteristic for a specific tumor entity rather than a particular age group were gains of 9p for anaplastic astrocytomas (20% of pediatric and 14% of adult anaplastic astrocytomas versus 0 and 5% for glioblastomas) and losses of 13q, containing the retinoblastoma tumor suppressor gene, for glioblastomas (31% of pediatric and 38% of adult glioblastomas versus 10 and 23% for anaplastic astrocytomas). Furthermore, characteristic aberrations distinguishing the two tumor grades within the pediatric age group consisted of gains of 5q and losses of 9q, 12q, and 22q for anaplastic astrocytomas as well as gains of 1q, 3q, and 16p and losses of 8q and 17p for glioblastomas. Loss of 22q is a frequent event in several tumor entities, eg, ependymomas, pheochromocytomas, rhabdoid tumors, and hepatocellular, colorectal, ovarian, breast, and oral squamous cell carcinomas, and was observed in 17, 31, and 38% of adult low-grade, anaplastic astrocytomas and glioblastomas, respectively, consistent with a role in astrocytoma tumorigenesis and progression.³⁵ However, among our pediatric cases loss of 22q was most frequently encountered in anaplastic astrocytomas rather than glioblastomas (Figure 1). Moreover, amplifications of CDK4 (12q), CCND1 (11q13), and CCND3 (6p21) that have been found in adult malignant gliomas³⁶ do not seem to play a role among pediatric high-grade astrocytomas (Figure 1). Of the minimal common regions found in our series most are novel and have not previously been put forward. They consisted of gains of 1q21-41 (containing the proto-oncogenes SK1, PBX1, TRK/TRKC, ABL, and ELK4), 2q31-32, 3q27-qter (LAZ3/MLF1), and 5q14-22 (GAP) as well as losses of 6q25-qter (containing the tumor suppressor genes MAS and

IGF2R), 9q34.2 (TSC1), 10q23-25 (PTEN), 11q14-22, 16q22-qter (CMAR/CAR and E-cadherin), 17p (TP53 and OVCA1/2), and 22q12-qter (BAM22 and NF2). Furthermore, the whole q-arm of chromosome 10 was lost in five cases, indicating that not only PTEN but additional putative tumor suppressor genes may be involved in the development of glioblastomas, eg, DMBT1, FGFR2, and/or MXI1 that are located at the distal end of 10q.³⁷⁻³⁹ Apart from PTEN, DMBT1, FGFR2, and MXI1 at 10q, TP53 at 17p13.1 and NF2 at 22q12, none of the known tumor oncogenes or suppressor genes mentioned above have yet been associated with astrocytoma tumorigenesis.

Tumor Proliferation, Genetics, and Prognosis

MIB-1 proliferation indices in our series were significantly lower in anaplastic astrocytomas than in glioblastomas corroborating previous investigations.^{40,41} A significantly shorter survival was found for all high-grade astrocytomas with a MIB-1 proliferation index of >25% compared with tumors with a MIB-1 proliferation index of <25% reflecting a similar difference in outcome presented for MIB-1 proliferation cut-off levels of 11%⁴¹ and 12%.⁴⁰ Although age was not prognostically significant, the three youngest children in this series which were <2 years of age were still alive 8, 27, and 107 months after diagnosis. The 1-, 2-, and 3-year survival rates overall in our series were 55, 33, and 21%, respectively, which are similar to the 2- and 3-year survival rates of 43% and 35 to 36% encountered elsewhere.⁴²⁻⁴⁴ Outcome was significantly better for anaplastic astrocytomas with 1-, 2-, and 3-year survival rates of 70, 60, and 50%, respectively, compared with 46, 9, and 0% for pediatric glioblastomas.

When correlating prognosis with specific chromosomal aberrations, a significantly shorter survival among children with anaplastic astrocytomas showing gains of 1q compared to cases without this change could be found; this correlation could not be established for pediatric glioblastomas (Figure 2). Although an association between TP53 mutation and a shorter progression-free survival has been proposed in one investigation on pediatric malignant gliomas,⁴⁵ no association between presence of TP53 mutation and patient survival was found in two series of adult glioblastomas;^{8,46} in our study, the five children with -17p all died between 4 and 15 months after diagnosis. Correlations between prognosis, tumor dynamics and chromosomal imbalances have previously been presented for adult high-grade gliomas and consisted of more frequent gains of 12q14-21 and 19 among slower growing glioblastomas compared with gains of 6q16-qter, 13, and 20 among faster growing subgroup.²⁶ Another study found higher chemosensitivity, longer recurrence-free survival after chemotherapy, and longer overall survival among anaplastic oligodendrogliomas to be associated with loss of chromosomes 1p and 19q whereas CDKN2A gene deletions had a worse prognosis.⁴⁷ On the other hand, loss of the INK4a-ARF locus on chromosome 9p21 and loss of 14pARF expression was speculated to contribute to the highly malignant behavior and treatment resistance of adult high-grade astrocyto-

mas;⁴⁸ loss of material on 9p, however, only affected two children with anaplastic astrocytomas, who were still alive at time of reporting, and one with glioblastoma who died after 12 months.

In conclusion, our results show that although there may be some common genetic steps in the malignant progression of both pediatric and adult astrocytomas there are also a number of nonrandom aberrations that have not been observed in adult high-grade astrocytomas, supporting a different genetic pathway and contradicting the notion that high-grade astrocytic tumors in children differ from those in adults by lacking consistent numerical and structural deviations.¹⁰ Furthermore, gains of chromosomal material on 1q seem to be correlated with a worse prognosis among pediatric anaplastic astrocytomas but not glioblastomas. However, as these histological groups consisted of only 10 and 13 cases, respectively, this finding will have to be corroborated among a larger cohort.

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