

## Recurrent Gain of Chromosome Arm 7q in Low-Grade Astrocytic Tumors Studied by Comparative Genomic Hybridization

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Consistent tumor-specific chromosomal aberrations have not been described in low-grade astrocytic tumors. The most frequent genetic alterations are mutations of the *TP53* tumor suppressor gene and/or loss of heterozygosity (LOH) on 17p that occur in about 30% of the cases in adult patients but that are uncommon in childhood tumors. We used comparative genomic hybridization (CGH) to map DNA copy number alterations in 18 primary low-grade astrocytic tumors (ten adult patients and eight children). A gain of chromosome arm 7q was the most frequent event detected in five of ten astrocytomas (50%) from adult patients, followed by DNA amplification on chromosome arm 8q and gain on 12p (two cases). Loss of chromosomal regions on 1p, 4q, and the X chromosome was observed in two of ten cases each [including one patient afflicted with Turner syndrome (45,X)]. In contrast, no consistent changes were observed in low-grade astrocytomas in children. A loss of the X chromosome was the sole aberration detected in two of eight cases using DNA extracted from normal brain tissue. The findings suggest that a gain of 7q is an early event in the initiation of astrocytomas in adult patients. The discrepant findings in low-grade astrocytic tumors in adults compared to tumors in children support the hypothesis that there might be different mechanisms responsible for tumor development. *Genes Chromosom Cancer* 15:199-205 (1996). © 1996 Wiley-Liss, Inc.

### INTRODUCTION

Astrocytic tumors are the most common brain tumors both in children and adults (de Vita et al., 1993). The World Health Organization (WHO) classification defines four different groups of astrocytomas (Kleihues et al., 1993). Pilocytic astrocytomas (grade 1) comprise a distinct tumor entity. Astrocytic tumors can progress in an individual patient from grade 2 to grade 4. Low-grade astrocytomas (grade 2) are composed of well-differentiated neoplastic astrocytes. Anaplastic astrocytomas (grade 3) show focal or diffuse anaplasia, whereas glioblastomas (grade 4) contain poorly differentiated cells and show vascular proliferation and/or necrosis. The biological behavior of these tumors is determined by creating a space-occupying mass, by infiltration into vital structures, and by resistance to radiation and chemotherapy. Their potential to recur is high, whereas metastases are rare. Astrocytic tumors in children differ from tumors of the same stage in adults with respect to the localization and the prognosis, which is better in children (Kleihues et al., 1993).

Consistent tumor-specific aberrations were revealed in anaplastic astrocytomas and glioblastomas using karyotype analysis, loss of heterozygosity (LOH) studies, gene expression studies (for re-

view, see Batra et al., 1994, and references cited therein), and DNA fingerprinting (Nürnberg et al., 1991). By using comparative genomic hybridization (CGH), we could delineate recurrent chromosomal gains and losses in high-grade astrocytic tumors (Schröck et al., 1994). Our findings were confirmed subsequently by Kim et al. (1995), using an identical experimental approach.

Very few cytogenetic data on low-grade astrocytomas have been reported on in the literature (Mitelman, 1994). Among mostly normal karyotypes, sex chromosome loss was detected in about 20% of the cases, whereas structural aberrations of chromosome 1 and a trisomy of chromosome 7 were reported as rare clonal events.

CGH (Kallioniemi et al., 1992; du Manoir et al., 1993) is a comprehensive screening test for copy number changes in test genomes and has been proven to identify genomic imbalances in a variety of solid tumors, including high-grade astrocytic tumors (Schröck et al., 1994; Kim et al., 1995). We used CGH to analyze chromosomal imbalances in

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TABLE 1. Clinical Data of Patients, Pathological Data of the Primary Low-Grade Astrocytic Tumors (A2) in Children, Karyotypes, and CGH Results<sup>a</sup>

Lab. no.	Age in years/sex	Localization	Clinical course <sup>b</sup>	Karyotype <sup>c</sup>	CGH results
159	2/Female	Cerebellum	Symptoms and duration of symptoms unknown; four recurrences after surgery every 6 months, with increasing stage of malignancy up to A4; dead	46,XX[20]/92,XXXX[4]	+9, +11, +19
177	10/Female	Cerebellum	Headache for 2 years before surgery; no recurrence; present status unknown	87-96,XXXX, +4, +5, +6, +7, +7[3]/65-84, XXX, -X[6], -1[3], -2[9], +4[2], -8[4], -17[3], -18[5] [cp10]46,XX[4]	-2
183	4/Female	Cerebellum	Headache for 1 month; surgery 1 year ago; alive	42-46,XX[55]	No gain, no loss
206	16/Male	Left temporal	Epilepsy for 2 months before surgery; no recurrence; present status unknown	47,XY, +7[1]/46,XY[12]	No gain, no loss
295	8/Female	Hippocampus, left temporal	Epilepsy for 4 years; surgery 2 years ago; alive	46,XX[36]	No gain, no loss
297	7/Male	Cerebellum	Learning problems and ataxia; duration of symptoms unknown; no recurrence; alive 2 years after surgery	46,XY[12]	No gain, no loss
365	15/Male	Hippocampus, left	Epilepsy for 2 years before surgery; no recurrence; alive 1 year after surgery	46,XY[27]	No gain, no loss
380	9/Female	Thalamus, left	Headache for 6 months before surgery; learning disability; no recurrence; alive 1 year after surgery	46,XX[52]	-22

<sup>a</sup>CGH, comparative genomic hybridization.

<sup>b</sup>The data for the clinical course were prepared in October, 1994.

<sup>c</sup>Karyotypes of cases 177, 183, and 206 were published in Thiel et al., 1993.

18 low-grade astrocytomas (grade 2) that had been studied previously by chromosome banding. A gain of 7q was detected by CGH in 50% of the tumors in adult patients.

## MATERIALS AND METHODS

### Normal and Tumor Brain Tissue

Normal brain tissue from eight adult patients who died from nonneurological causes was collected at autopsy. The ages of the four male and four female patients ranged from 58-79 years, and material was collected from the cerebral cortex.

Clinical and histopathological data, CGH results, and karyotypes of 18 low-grade astrocytomas (grade 2) are presented separately for children (eight cases) and adults (ten cases) in Tables 1 and 2, respectively. Brain tumor material was obtained during surgery and was used for cell culture, for

DNA preparations, and for formalin fixation. The histopathological diagnosis of paraffin-embedded tissue was performed according to the WHO classification (Kleihues et al., 1993). In each case, the most recent follow-up data of the clinical course (as of October, 1994) were obtained from the treating physician.

### Karyotype Analysis

Metaphase chromosomes from primary cell cultures of all tumor samples were prepared and analyzed as described (Thiel et al., 1992).

### CGH

Tumor DNA was extracted from frozen tissue, normal brain DNA was prepared from autopsy material, and reference genomic DNA was extracted from peripheral blood lymphocytes of a healthy

TABLE 2. Clinical Data of Patients, Pathological Data of the Primary Low-Grade Astrocytic Tumors (A2) in Adult Patients, Karyotypes, and CGH Results

Lab. no.	Age in years/sex	Localization	Clinical course <sup>a</sup>	Karyotype <sup>b</sup>	CGH results
82	29/Female	Right precentral	Epilepsy for 7 months before surgery; recurrence 16 months after surgery; alive 3 years after surgery	47,XX,+7,+dmin[3]/40-46,XX[5]	+ (7)(q21-qter), ampl.(8)(q21-qter), + (10p), + (12p)
96	39/Female	Temporofrontal	Epilepsy for 6 months before surgery; recurrence 22 months after surgery; present status unknown	45,X,-X[6]/46,XX[29]	-4q,+ (7)(pter-p12), + (7)(q21-qter), amp.(8)(q23-qter), + (12)(pter-p12), -X
113	21/Male	Right temporal	Epilepsy for 13 years before surgery; recurrence 5 months after surgery; present status unknown	46,XY[46]	-Y
180	30/Male	Left temporofrontal	Epilepsy for 1 year before surgery; present status unknown	46,XY[26]	+7
194	32/Female	Left temporooccipital	Headache for 17 years before surgery; epilepsy for 2 months before surgery; recurrence 2 years after surgery; alive 3 years after surgery	45,X [24] (Turner syndrome)	+ (7)(q21-qter), ...X
197	21/Female	Frontal	Epilepsy before surgery; duration unknown; recurrence 14 months after surgery; present status unknown	44-46,XX[13]	No gain, no loss
200	56/Male	Brainstem	Symptoms and duration of symptoms unknown; death 4 months after surgery	84-90,XXYY,del(1)(q21)x2,der(2)t(1;2;?)(q23;p13;?)x2,+ der(2)t(2;7)(q37;q22)x2,add(5)(q35)x2,-6,add(6)(q23)(6pter-6q23::?),-8,del(11)(q22),-13,der(15)t(2;15)(perq;q26),-16,-18,+1-4mar[22]	+ (7)(q22-qter), -(11)(q22-24), -18
269	54/Female	Intraventricular	Resection of a meningioma (localization, frontal) 5 years before surgery due to astrocytoma; no recurrence; alive 2 years after surgery	46,XX[22]	No gain, no loss
304	33/Female	Left frontal	Single grand mal 1 month before surgery; present status unknown	46,XX[29]	-(1p)
367	64/Female	Left central parietal	Epilepsy for 22 years before surgery; alive 1 year after surgery	46,XX[19]/44-45,XX[4]	-(1p), -4, -(9)(pter-p13), -(19q)

<sup>a</sup>The data for the clinical course were prepared in October, 1994.

<sup>b</sup>Karyotypes of cases 82, 96, 113, 180, 194, 197, and 200 were published in Thiel et al., 1993.

male donor (46,XY) by using standard procedures. The tumor DNA and the DNA prepared from normal brain were labeled with biotin-16-dUTP (Boehringer Mannheim, Indianapolis, IN), and the reference DNA was labeled with digoxigenin-11-

dUTP (Boehringer Mannheim) by nick translation. An average fragment size of about 500 bp was achieved by adjusting the concentration of DNase I. The hybridization on reference metaphase cells prepared from lymphocytes of healthy male donors

and the detection were performed as described (Ried et al., 1994; Schröck et al., 1994).

#### Digital Image Acquisition and Analysis

Digital gray-scale images were recorded subsequently for FITC, rhodamine, and DAPI by using a cooled charge-coupled device (CCD) camera (Photometrics, AZ) connected to an epifluorescence microscope (Zeiss, Federal Republic of Germany; Axiophot and Leica, Federal Republic of Germany; DMRBE). The G-like banding pattern of the reference metaphase cells obtained by DAPI counterstaining was used for chromosome identification. Ratio images and average ratio profiles of ten metaphase cells per case were calculated by using the software and the procedure detailed by du Manoir and coauthors (du Manoir et al., 1995). Thresholds for gains and losses were defined as the theoretical value that would be expected in a diploid tumor cell population for a trisomy (1.25) or monosomy (0.75) of a certain chromosome in 50% of the test cells.

#### RESULTS

We analyzed 18 cases of low-grade astrocytic tumors (grade 2) that included ten tumors from adult patients and eight tumors from children by using karyotype analysis and CGH. For a control, we investigated eight cases of normal brain tissue obtained from autopsy material by CGH.

CGH did not reveal any copy number changes of autosomal chromosomes in DNA extracted from normal brain. A loss of the X chromosome was observed in one female (case B51) and in one male patient (case K56).

Chromosome banding studies revealed no recurrent aberrations in childhood tumors and tumors from adult patients in the cases included in our study (Tables 1, 2; Thiel et al., 1993). By using CGH, however, we detected notable differences in low-grade astrocytic tumors from children compared to adult patients. Copy number changes were rare in DNA extracted from children's tumors: Five of eight cases showed no imbalances. A gain of chromosomes 9, 11, and 19 or a loss of chromosomes 2 and 22 was detected in single cases. The combined results on childhood tumors are presented in Table 1.

Normal karyotypes were obtained for six of ten tumors from adult patients. A gain of chromosome 7 and dmns were detected in three of eight metaphase cells in case 82, whereas a complex karyotype was observed in case 200. Two cases showed a loss of the X chromosome as the sole aberration.

One of those patients (case 194) was previously diagnosed with Turner syndrome (45,X).

CGH revealed a gain of the entire chromosome or partial gains of the long arm of chromosome 7 in five of ten tumor samples from adult patients. Figure 1 shows a ratio image of one metaphase cell and the average ratio profile of ten metaphase cells from case 194. Data for all tumors from adult patients are summarized in Figure 2 and in Table 2. High-level copy number increases were mapped on chromosome arm 8q in two cases (82 and 96). Gain of 12p and losses of 1p, 4q, and the X chromosome (including the patient with Turner syndrome, case 194) were observed in two cases each. The Y chromosome was lost in one male patient. Whole arm gain was observed once on 10p. Chromosome 18 was lost entirely in one case, and partial losses were mapped to 9p, 11q, and chromosome 19 in individual patients. Two cases showed normal average ratio profiles for all chromosomes.

#### DISCUSSION

We have previously reported CGH results from nine cases of high-grade astrocytic tumors (Schröck et al., 1994). The most consistent finding was a gain of chromosome 7 as well as a loss of chromosome 10. Localized, high-level copy number increases (amplifications) were also frequently observed. These results were consistent with data obtained by karyotype analysis, LOH studies, and molecular biological studies of gene amplification.

Far less is known about chromosomal aberrations in low-grade astrocytic tumors, which have a strong tendency for local recurrence and progress to high-grade tumors in the same patient. Therefore, the delineation of genomic alterations in low-grade astrocytic tumors would offer insight into early changes that occur during the genesis of glial tumors.

The number of chromosomal aberrations in low-grade astrocytic tumors detected by CGH is low compared to anaplastic astrocytomas and glioblastomas. We mapped 88 copy number changes in nine cases of high-grade astrocytic tumors, whereas the number of chromosomal aberrations in ten low-grade astrocytomas was 22. Only chromosome 7 was subject to copy number changes in both tumor groups. Eight of nine high-grade tumors revealed a gain of sequences on chromosome 7, whereas a gain of the long arm of chromosome 7 was observed in five of ten grade 2 astrocytomas. Therefore, we conclude that the increased expression of genes on chromosome 7 provides an important growth advantage to glial cells that is followed by additional

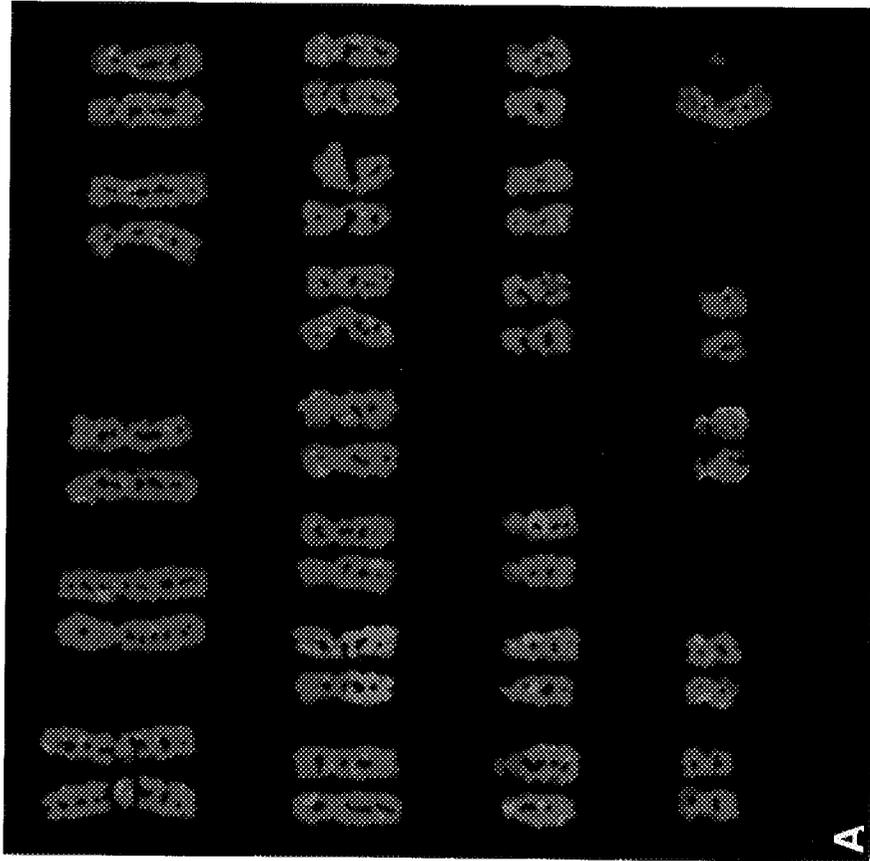


Figure 1. Evaluation of the comparative genomic hybridization (CGH) experiment using tumor DNA (FITC fluorescence) of a low-grade astrocytic tumor from an adult patient (case 194, female) and control DNA (rhodamine fluorescence) prepared from lymphocytes of a healthy male donor. **A:** Fluorescence ratio image. A three-color look-up table was chosen to visualize the pixel-by-pixel FITC/rhodamine ratios. Blue color indicates a balanced state between tumor and control DNA, green demonstrates an overrepresentation of DNA sequences in the tumor genome, and red indicates a loss at the corresponding regions in the tumor. Note the gain on chromosome arm 7q. The blue color of the X chromosome indicates a balanced state between the female tumor DNA and the male control DNA, which is consistent with the cytogenetic finding of a loss of the X chromosome in a female patient afflicted with Turner syndrome (45,X). **B:** Average ratio profile calculated from individual profiles of ten metaphase

cells using a computer program for the quantitative analysis of fluorescence images (du Manoir et al., 1995). The three vertical lines on the right side of the chromosome ideogram indicate the normal range. The left line represents the threshold for a loss (value 0.75, corresponding to a monosomy in 50% of the cells in a diploid tumor), and the right line represents the threshold for a gain (value 1.25, corresponding to a trisomy in 50% of the cells in a diploid tumor) of DNA sequences in the tumor genome. The gain of chromosome region 7q21-qter is clearly visible. The ratio value for a balanced state of the X chromosome using female tumor DNA vs. male control DNA is expected to be 2.0. The profile demonstrates a value between 0.75 and 1.0, indicating a loss of the X chromosome, which is consistent with the finding of a 45,X karyotype in a patient with Turner syndrome and astrocytoma.



Figure 2. Summary of chromosomal imbalances in ten cases of low-grade astrocytic tumors from adult patients detected by CGH. High copy number increases (amplifications; bars), gains (lines on the right of the chromosome scheme), and losses (lines on the left of the chromosome scheme) are displayed. Overrepresentation of chromosome 7 was detected in 50% of the cases, and two tumors revealed amplifications on chromosome 8q. The individual case numbers are shown next to the lines.

changes, e.g., the loss of putative tumor suppressor genes on chromosome 10, which is almost invariably lost in glioblastomas. Regional, high-level copy number increases that were often observed and mapped to chromosome 7 in high-grade astrocytic tumors (including the amplification of the *EGFR* gene) are obviously late events in tumor progression. However, we detected high-level copy number increases in low-grade astrocytomas on the long arm of chromosome 8 (the region that contains the *MYC* protooncogene), although amplifications are rare events in glioblastomas.

We did not observe decreased copy numbers for 17p (the location of the *TP53* tumor suppressor gene), although LOH at 17p is a recurrent finding in low-grade astrocytomas (von Deimling et al., 1992, 1994; Rasheed et al., 1994). Further studies of astrocytic tumors, therefore, should take advantage of a combined approach using mutation analysis of the *TP53* tumor suppressor gene and CGH.

Karyotype analysis performed for all cases revealed no specific chromosomal aberration. A gain of a part or the entire chromosome 7 was detected in only a small number of cells in two adult patients (82 and 200) compared to the finding in five cases (82, 96, 180, 194, and 200) using CGH. The banding results were confirmed in most of the cases, and a higher frequency of chromosomal aberrations was detected using CGH.

Astrocytic tumors in children, compared to adults, show a different location, a different growth pattern, longer survival times, and a lesser tendency to recur. No specific chromosomal aberrations (Thiel et al., 1993), *TP53* gene alterations, LOH, or gene amplification (Rasheed et al., 1994) were detected. In contrast to the CGH data in low-grade astrocytic tumors from adult patients, childhood tumors revealed no recurrent chromosomal imbalance using CGH, and karyotypes were normal in most of the cases.

The biological significance of a gain of chromosome 7 in tumors is disputed in the scientific literature. To determine whether normal brain tissue shows any chromosomal imbalance, we used CGH to study autopsy material from eight patients who died from heart attack or other reasons. Neither a brain tumor nor any brain disease was diagnosed in these patients. A loss of the X chromosome was observed in two cases. Copy number changes of autosomal chromosomes were not detected. There-

fore, we conclude that the complete or partial gain of chromosome 7 is tumor-related rather than tissue-related.

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