

## CYTOGENETIC INVESTIGATION OF FOUR LOW GRADE GLIOMAS

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### ABSTRACT

Four low-grade gliomas - two oligodendrogliomas and two astrocytomas - were analyzed cytogenetically. All cases exhibited monosomies of chromosomes 10 and 11. The astrocytomas shared monosomies of chromosomes 8, 9, 10, 11, 12, 18 and 20. Losses of chromosomes 3, 5, 6, 10 and 11 were present in both oligodendrogliomas, and except for monosomy of chromosome 6, were also identified in the pilocytic astrocytoma.

### INTRODUCTION

Gliomas are the most common primary tumors of the human central nervous system. They are classified histologically into low-grade (grade I-II astrocytomas, oligodendrogliomas and ependimomas), and high grade or malignant (anaplastic astrocytoma and glioblastoma multiforme) (Haskell, 1990).

The cytogenetic alterations most frequently found in low grade gliomas are monosomies of chromosomes 10, 22 and the sex chromosomes, gains of chromosome 7 and structural abnormalities of 1p, 8p, 9p and deletions of 7p (Bigner *et al.*, 1984, 1990; Griffin *et al.*, 1988; Jenkins *et al.*, 1989; Rey *et al.*, 1987b; Rogatto *et al.*, 1989; Shapiro *et al.*, 1985). Rogatto and Casartelli (1988a) found that the chromosome region 2q24-2q32 was involved in rearrangements in six low-grade gliomas. Structural aberrations of 1p, 11q and deletions of 6q have been reported by Rey *et al.* (1987b) and Jenkins *et al.* (1989). According to Mitelman *et al.* (1990), chromosome regions 7p22-q36, 10p15-25 and 22p13-q13 are considered to be involved in the primary events in the genesis of astrocytomas. Thiel *et al.* (1992) reported trisomy of chromosome 7, monosomy of chromosome 20 and deletions of chromosome 22 as the main alterations found in twelve oligodendrogliomas.

We report here a cytogenetic study of four low-grade gliomas.

### MATERIAL AND METHODS

Table I summarizes the case number, age, sex and histologic diagnosis of the four patients in this study.

Table I - Summary of histopathological findings in the four cases analyzed.

Case no.	Age/Sex	Histological diagnosis
1	27/M	Oligodendroglioma
2	02/F	Oligodendroglioma
3	25/M	Grade II Astrocytoma
4	27/M	Pilocytic Astrocytoma

### Tissue culture and cytogenetics

Fragments of the surgical specimens, received under sterile conditions, were cut into very small pieces and plated in sterile bottles containing HAM-F10 medium (Sigma), supplemented with 20% fetal calf serum and antibiotics. Cells were grown at 37°C and fed twice a week. For cytogenetic analysis, cells in the exponential growth phase were treated with 0.0016% colchicine for at least three hours, collected with 0.05% trypsin, treated with hypotonic 0.075M KCl for about 20 minutes at 37°C and fixed with methanol-acetic acid (3:1). Metaphases were submitted to standard staining with Giemsa and banded with trypsin-Giemsa (G-banding).

Karyotypes and cytogenetic clones were described and defined according to the ISCN (1985, 1991).

**RESULTS**

*Case 1 (oligodendroglioma)*

Chromosome counts were performed on 113 cells. Two modal values, 45 and 46 chromosomes, were found. Eighty-two cells showed hypodiploid karyotypes and only eight cells were identified in the hyperdiploid range.

No. of chromosomes	23	25	30	31	32	34	36	37	38
No. of cells	1	2	1	3	3	2	3	1	6
	39	40	41	42	43	44	45	46	52
	3	8	5	16	3	1	24	23	1
	86	90	92	161					
	2	1	3	1					

Eight cells were analyzed by GTG banding (Table II). Monosomy of chromosome 3 was the only clonal numerical alteration determined in 50% of the cells analyzed. Non-clonal monosomies of chromosomes 5, 6, 10, 11 and 13 were determined in two cells each (Figure 1).

Table II - Detailed karyotypic findings of case 1.

Karyotype
40, XY, -4, -6, -7, -8, -10, -13
42, XY, -1, -5, -6, -10, -13, +2 mar?
43, XY, -3, -5, -11, +19, -22
43, XY, -3, -9, -11
44, XO, -Y, +6, del(9p), del(10p), -11, -22
45, XY, -3
91, XXYY, -15
161, XXXXXYYY

*Case 2 (oligodendroglioma)*

Chromosome counts were performed on 111 metaphases. The modal chromosome number found was 46. Eighty-one cells were in the hyperdiploid range.

No. of chromosomes	19	22	23	24	25	26	28	29	30
No. of cells	1	1	2	2	1	1	1	3	2
	31	32	33	34	35	36	37	38	39
	2	2	1	4	1	1	2	9	4
	40	41	42	43	44	45	46	47	48
	1	5	18	1	7	9	20	1	1
	54	55	57	60	> 66	72	86		
	1	1	1	1	1	1	2		

Ten cells submitted to GTG banding were analyzed (Table III). Monosomy of chromosome X was the most frequent numerical alteration found in this case (70% of the cells). Monosomy of chromosome 1 was determined in 60% of the cells analyzed and loss of chromosome 21 was detected in 50% of the cells. In 40% of the cells there were monosomies of chromosomes 4 and 6, and in 30% of the cells there were losses of chromosomes 3, 9, 12, 15, 17, 19 and 22 respectively.

The only structural clonal aberration found was 18p- in 50% of the cells analyzed. Non-clonal numerical monosomies of chromosomes 5, 8, 10, 11 and 16 were also determined.

*Case 3 (astrocytoma grade II)*

Chromosome counts were made on 129 cells. The modal chromosome number was 46. Chromosome numbers ranged from 20 to 96 chromosomes per cell. Sixty-seven cells in the hypodiploid range and 27 cells in the hyperdiploid range, were identified.

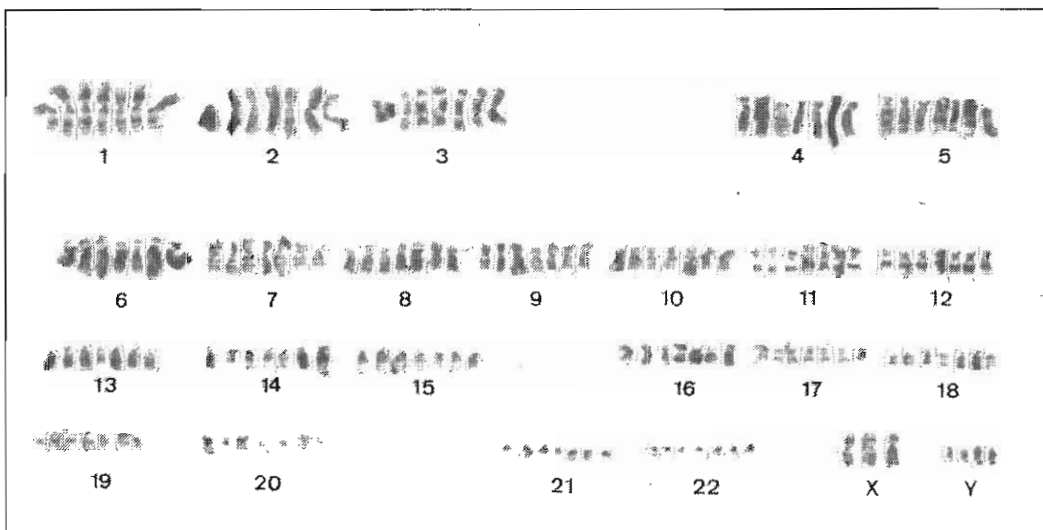


Figure 1 - Karyotype of a cell of patient 1, with 161 chromosomes.

Table III - Detailed karyotypic findings of case 2.

Karyotype	
35, XX, -1, -3, -6, -8, -9, -12, -17, -18, -19, -20, -22	
37, XO, -X, -1, -2, -6, -10, -11, -16, -17, -21	
37, XO, -X, -1, -3, -4, -6, -12, -12, -20, -21, -22, +mar?	
39, OO, -X, -X, 4, -4, -9, -10, -18, 22q-	
39, OO, -X, -X, -1, -3, -11, -13, -15, 18p-, -21, +mar?	
40, XO, -X, -6, -16, -17, -17, -17, +18, 18p-, -19, -21, -22, +mar?	
41, XO, -X, -1, del (3q), -5, -8, -12, 18p-	
41, XO, -X, -1, -4, -4, -5, -14, -15, +16, +mar?	
43, XX, -9, -15, -19, -21, +mar?	
45, XX, -4, 18p-	

No. of chromosomes	20	23	24	25	29	30	31	33	34
No. of cells	1	2	1	1	2	4	1	1	1

36	37	38	39	40	41	42	43	44
2	1	7	2	2	3	2	11	14

45	46	47	48	49	51	52	75	76
9	35	4	5	1	1	1	1	1

86	88	90	92	94	95	96
1	1	1	6	1	1	2

Table IV - Detailed karyotypic findings of case 3.

Karyotype
30, XY, +1, +4, +5, +6, +7, +8, +9, -10, +11, -20, -21
36, XO, -X, -1, -2, -8, -9, -14, -15, -16, -17, -22
41, XY, -6, -8, -11, -12, -18
41, XO, -Y, -8, -9, -11, -12, +14, -16
43, XY, del(2)(p24), -15, -19, -20
43, XO, -Y, -16, -21
44, XO, -Y, del(2)(p24), del(6)(p21), -7, -10, +18, 18p+, -19, +22
44, XY, -4, +14, -18, -19
44, XO, -Y, -1, del(2)(p24), del(6)(p21)
45, XY, -9, +21, -22
47, XY, +mar?
76, XY, -X, +1, -2, +3, +5, +6, +7, -9, +11, +15, +16, +21, +22
89, XXXY, -3, -10, -13

Thirteen cells were submitted to GTG banding (Table IV). Clonal numerical alterations found in this case were monosomies of chromosomes Y, 8, 9, 16 and 19 in 30% of the cells. Trisomy of chromosome 14 was determined in 20% of the cells. The clonal structural alterations most frequently identified were the deletions del(2)(p24) in 30% of the cells, and del(6)(p21) in 20%. The simultaneous presence of both deletions in the same cell was identified in 20% of the cells karyotyped (Figure 2).

Non-clonal monosomies of chromosomes 2, 10, 11, 12, 15, 18, 20 and 22 were also found.

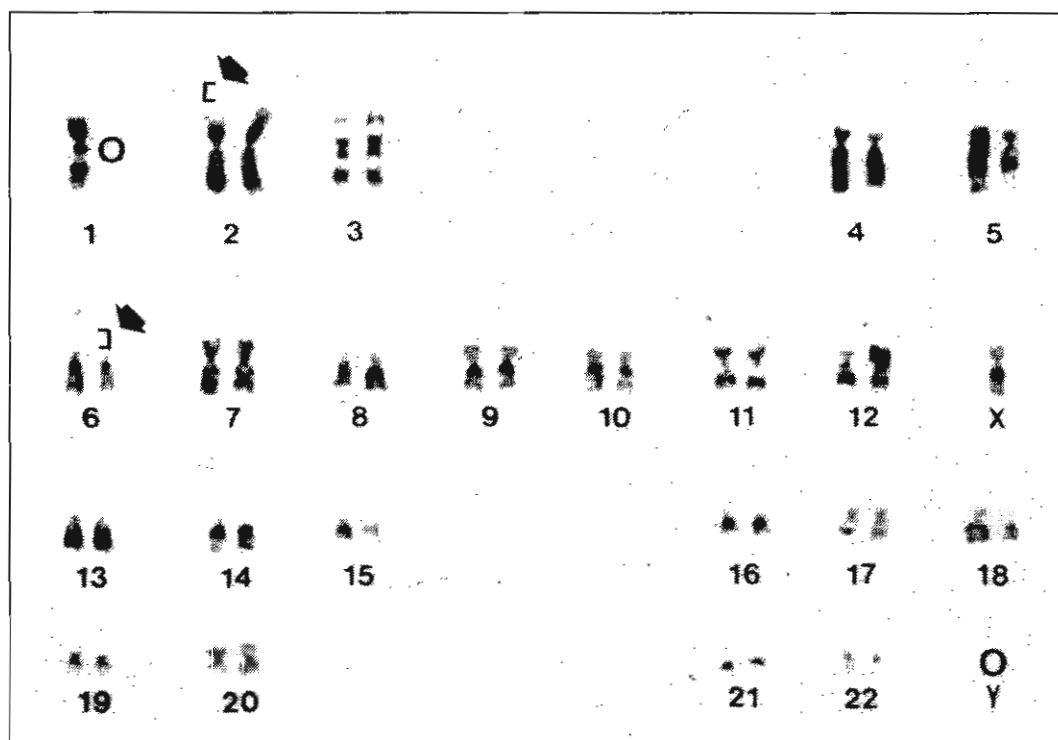


Figure 2 - Karyotype of a cell with 44 chromosomes with the following deletions: del(2)(p24) and del(6)(p21).

Note also the monosomies of chromosomes 1 and Y.

Case 4 (pilocytic astrocytoma)

Chromosome counts were performed on 105 cells. The modal chromosome number determined was 46 (29.5%). Karyotypic variation ranged from 30 to 94 chromosomes per cell; 28.6% of the cells were in the hypodiploid range and 41.9% were hyperdiploid.

No. of chromosomes	30	31	36	38	39	43	44	45	46
No. of cells	1	2	1	3	1	1	4	17	31
	47	48	52	66	78	84	86	87	88
	6	3	1	1	1	2	1	2	1
	90	92	93	94					
	1	22	1	3					

Nine cells were analyzed by GTG banding and the corresponding results are shown in Table V.

Table V - Detailed karyotypic findings of case 4.

Karyotype
41,XY,-8,-11,-12,-12,-17,-20,+mar?
42,XY,-10,-12,-15,-21
44,XY,-6,-18
45,XY,-10
45,XY,-10,-22
46,XY
48,XY,+2 mar?
92,XXYY,-10,-13,+15,+16
92,XXYY

The only clonal numerical alteration found was monosomy of chromosome 10. No structural alterations were found. Other chromosomes were sporadically involved in monosomies and were not considered to be clonal numerical alterations since they were present in one cell only (Figure 3).

DISCUSSION

Oligodendrogliomas

Both oligodendrogliomas analyzed here showed clonal monosomy of chromosome 3 (which was the only clonal alteration in case 1). The lack of evidence in the literature of involvement of this chromosome in oligodendrogliomas may be due to the fact that this is a secondary casual event or perhaps that this is a glioma subtype, although more cases need to be studied in order to confirm this hypothesis.

Gonosomal losses have been reported previously in association with oligodendrogliomas by Rey *et al.* (1987b) and Jenkins *et al.* (1989). In case 2 we found monosomy X in 70% of the cells analyzed, in agreement with Thiel *et al.* (1992) who often identified losses of this chromosome in twelve oligodendrogliomas studied. Clonal monosomies of chromosomes 1, 4, 6 and 21 were also found (case 2) and in both cases (cases 1 and 2) there were non-clonal alterations: -5, -10 and -11. The involvement of these chromosomes in human gliomas has been previously reported by several authors (Rey *et al.*, 1987b; Bigner *et al.*, 1984, 1986; Jenkins *et al.*, 1989).

The only clonal structural alteration found was 18p- which has also been identified by us in two

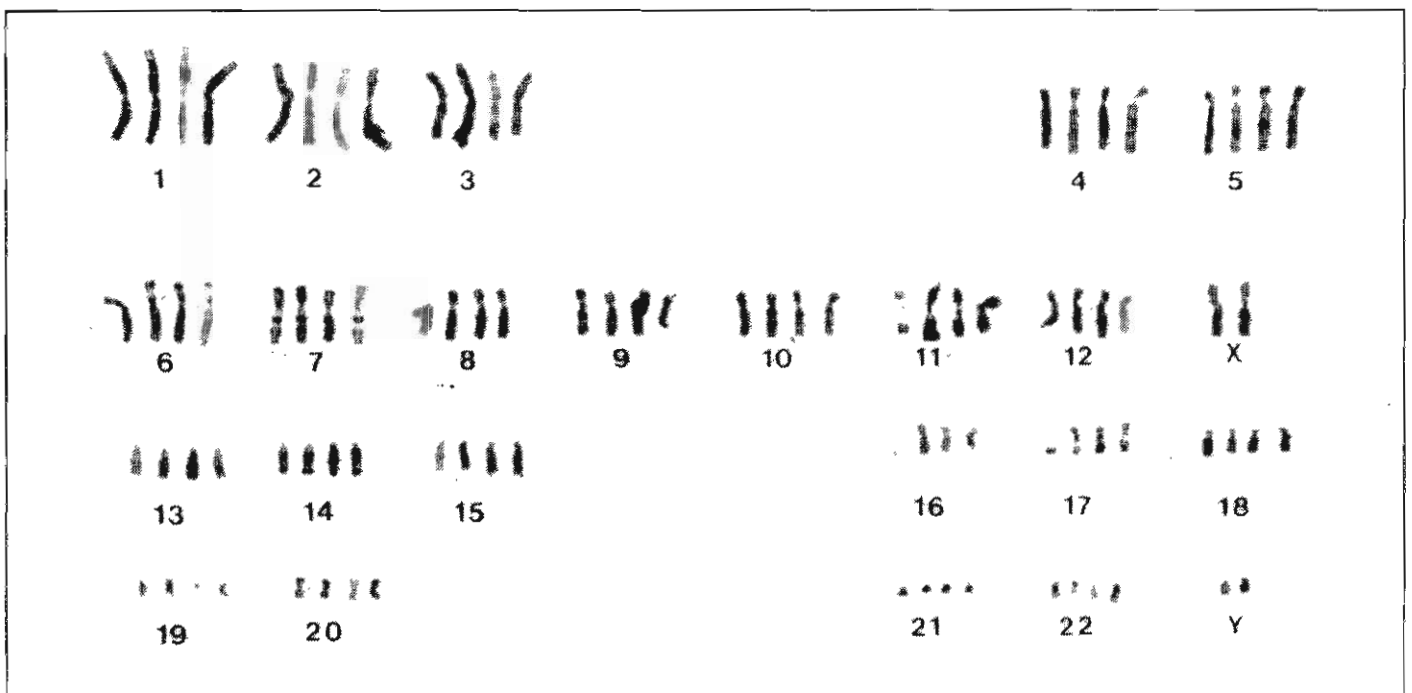


Figure 3 - Karyotype of a cell of patient 4, with 92 chromosomes.

glioblastomas multiformes (unpublished data). Jenkins *et al.* (1989) identified monosomy of chromosome 18 as the most common alteration in the series of 53 human gliomas they analyzed (diffuse astrocytomas, oligodendrogliomas, ependymomas, pilocytic astrocytomas, gliosarcomas and miscellaneous astrocytomas). Bigner *et al.* (1988) and Rogatto (1990) also identified this monosomy in glioblastomas multiformes. The presence of the same alteration in high and low-grade gliomas may indicate that chromosome 18 has genes involved in the genesis of human gliomas or this alteration may also indicate a progression to a more malignant stage.

### Astrocytomas

We analyzed one astrocytoma grande II and one pilocytic astrocytoma, and both bases exhibited clonal and non-clonal monosomies of chromosomes 8, 9, 10 and 15. Loss of chromosome 10 has been previously reported in low and high-grade gliomas (Bigner *et al.*, 1986, 1988; Rey *et al.*, 1987a,b; Jenkins *et al.*, 1989; Thiel *et al.*, 1992).

Aneuploidies of chromosomes 8 and 9 (cases 3 and 4) are not frequently associated with this neoplasia. Jenkins *et al.* (1989) reported anomalies of 8p and 9p associated with diffuse astrocytomas. Bigner *et al.* (1986) and Rey *et al.* (1987b) found alterations of the short arm of chromosome 9 in this neoplasia.

Deletions del(2)(p24) and del(6)(p21) were determined in the astrocytoma grade II (case 3). Rey *et al.* (1987a) identified structural rearrangements of chromosomes 1, 6, 7 and 9 as the main alterations found in 34 malignant human gliomas. Jenkins *et al.* (1989), analyzing three grade I and one grade II astrocytomas, found that chromosomes 2 and 6 were recurrently involved in monosomies and chromosome 6 was involved in structural rearrangements.

When we analyzed the clonal and non-clonal monosomies identified in all cases studied some coincidences were noted:

- All cases analyzed exhibited monosomies of chromosomes 10 and 11.

- Pilocytic (case 4) and grade II astrocytoma (case 3) shared monosomies of chromosomes 8, 9, 10, 11, 12, 18 and 20.

- Losses of chromosomes 3, 5, 6, 10 and 11 were present in both oligodendrogliomas (cases 1 and 2), and except for monosomy of chromosome 6, were also identified in the pilocytic astrocytoma.

According to Lasko *et al.* (1991), loss of heterozygosity is observed in glioblastoma, but not in lower grade tumors, presumably earlier on in a clonal progression. These investigators tried to infer a temporal order of genetic changes in brain tumors: loss of heterozygosity at 17p, amplification of EGF receptor/loss

of heterozygosity at 9p (grades III and IV), and finally, loss of chromosome 10 (grade IV only). However, we believe that this temporal order cannot be correct. Loss or structural abnormalities of chromosome 22 are the most common alterations in brain tumors, either low or high grade, and this was not mentioned by the authors (Al Saadi and Latimer, 1980; Bigner *et al.*, 1990; Couturier *et al.*, 1990; Mark and Levan, 1972; Rey *et al.*, 1987b,c, 1988; Rogatto and Casartelli, 1988a,b; Rogatto *et al.*, 1989; Seizinger *et al.*, 1986; Zankl and Zang, 1972). Losses of chromosome 9 or 9p were described by many authors in low-grade tumors and more specifically in low-grade gliomas, as well as losses or deletions of chromosome 10 (Rey *et al.*, 1987b; Griffin *et al.*, 1988; Rogatto *et al.*, 1989; Bigner *et al.*, 1990). Cytogenetic studies (Bigner *et al.*, 1988; Jenkins *et al.*, 1989) and molecular studies (James *et al.*, 1988; Fujimoto *et al.*, 1989; Fults *et al.*, 1990; Fults and Pedone, 1993; Watanabe *et al.*, 1990; Ransom *et al.*, 1992; Rasheed *et al.*, 1992) have shown that loss of sequences from chromosome 10 is a common genetic event in glioblastoma multiforme. Deletions or monosomies of chromosomes 9 and 10 (either clonally or non-clonally) are also present in almost all the cases described here, indicating that these alterations may occur early in the genesis of these tumors and could be related to proliferation instead of to malignancy. We believe that more molecular studies should be done on chromosome 10 in low grade gliomas in order to establish its role in the evolution of gliomas.

The analysis of coincidences or divergences in more cases may help to identify different glioma subtypes or common events in astrocytoma progression that may signal the transition from benign to malignant tumor stages. It will be necessary to investigate large series and to compare them by molecular, clinical and histopathological analysis in order to establish different glioma subtypes.

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### RESUMO

Quatro casos de gliomas de baixo grau de malignidade - dois oligodendrogliomas e dois astrocitomas - foram estudados citogeneticamente. Todos os casos analisados apresentaram monossomias dos cromossomos 10 e 11.

Os astrocitomas apresentaram em comum monossomias dos cromossomos 8, 9, 10, 11, 12, 18 e 20. Os oligodendrogliomas apresen-

taram perdas dos cromossomos 3, 5, 6, 10 e 11 e exceto pela monossomia do cromossomo 6, essas perdas foram comuns também ao astrocitoma pilocítico.

A análise de mais casos poderá ajudar a detectar diferentes sub-tipos de gliomas ou eventos comuns aos gliomas que pudessem indicar a transição de estágios benignos para malignos.

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