

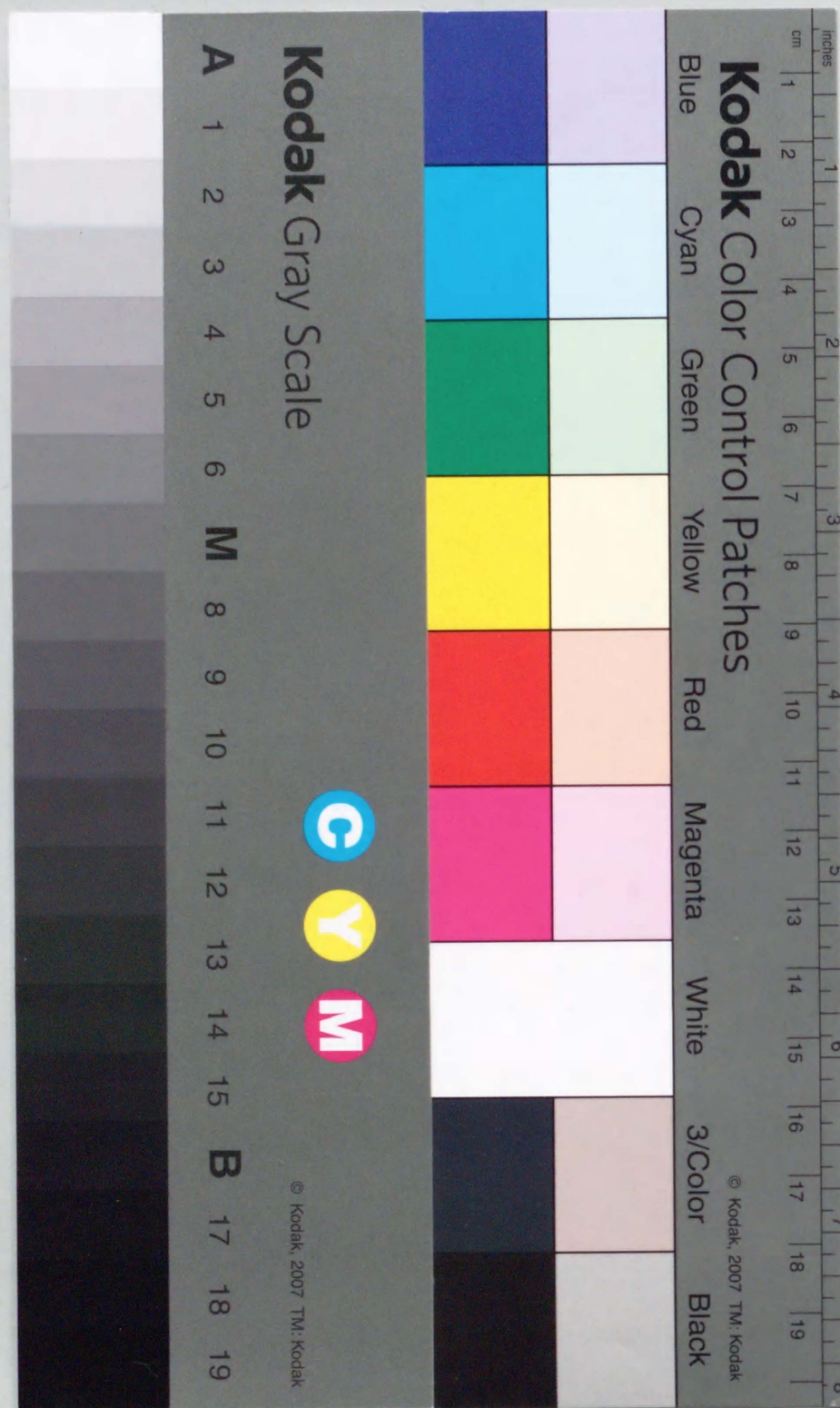


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Accumulation of allelic losses on chromosome 10 in human gliomas at recurrence

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Abstract

Aims—To elucidate the implications of allelic loss on chromosome 10 in the malignant progression of human gliomas.

Methods—Eight microsatellite loci (D10S249, D10S191, D10S210, D10S219, D10S246, D10S222, D10S221, and D10S212) were analysed for chromosomal deletions in histologically benign and malignant, including recurrent, gliomas. Of the 16 original tumours studied (two astrocytomas, nine anaplastic astrocytomas and five glioblastomas), the histological diagnosis at recurrence was anaplastic astrocytoma in six cases and glioblastoma in 10. Genomic DNA was extracted from formalin fixed, paraffin wax embedded sections. Samples of original and recurrent tumours were paired and amplified using PCR. Samples of histologically normal brain served as controls.

Results—Of the original tumours, all five glioblastomas, five (56%) of nine anaplastic astrocytomas and none of the astrocytomas demonstrated loss of heterozygosity (LOH) on chromosome 10. Additional LOH was detected in the five cases of anaplastic astrocytoma that progressed to glioblastoma at recurrence. Additional LOH was not detected in the two cases of astrocytoma that progressed to anaplastic astrocytoma at recurrence. With the exception of one case, additional LOH was observed in the recurrent glioblastomas.

Conclusion—LOH was observed at the loci of two adjacent microsatellite markers, D10S222 and D10S221 (10q23-q25), suggesting that this region on chromosome 10 is closely related to progression from anaplastic astrocytoma to glioblastoma.

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Keywords: chromosome 10, gliomas, loss of heterozygosity, microsatellite marker, recurrence.

The presence of fairly consistent chromosomal aberrations in human gliomas suggests that such genetic changes might be involved in neoplastic transformation or malignant progression of these tumours.¹⁻⁷ To date, the most frequent chromosomal changes seen in the most malignant glioma type—that is, glioblastoma, are loss of alleles on chromosome 10.⁸⁻¹² In many cases a large portion of this chromosome may be deleted.¹³⁻¹⁴ These findings suggest that there is a close relation between deletions on chromosome 10 and malignant progression in

gliomas. However, the loci deleted and the implications of these deletions have yet to be elucidated.

In the present study, loss of heterozygosity (LOH) was detected using multiple microsatellite markers. Special effort was made to elucidate the role of allelic losses in the malignant progression of gliomas. For this purpose, we examined paired samples of tumours obtained from the same patients at initial surgery and at recurrence.

Methods

Tissue samples were obtained from 16 patients with gliomas both at initial surgery and surgery following recurrence. Samples were fixed in 10% formalin and embedded in paraffin wax. Tumours were classified as recommended by the World Health Organisation (WHO),¹⁵ as astrocytoma in cases 1 and 2, anaplastic astrocytoma in cases 3-11 and glioblastoma in cases 12-16 at the initial surgery. At recurrence, the tumours were re-classified as anaplastic astrocytoma in cases 1-5 and glioblastoma in cases 6-16. The mean interval between the initial surgery and surgery for recurrence was 20.9 months (range 7-64 months).

EXTRACTION OF DNA

Paraffin wax sections, 6 µm thick, were stained with haematoxylin and eosin and the areas of tumour tissue were distinguished from adjacent brain tissue by microscopic examination. Genomic DNA was extracted from one to two adjacent sections, 10 µm thick. Normal brain and tumour tissue were separated carefully and processed separately for DNA extraction.

After deparaffinisation in xylene and 100% ethanol, the samples were digested in 100 mM Tris-HCl (pH 8.0), 50 mM EDTA (pH 8.0) and 10% SDS containing 100 mg/ml proteinase K at 55°C overnight. Lysates were extracted twice with phenol/chloroform (pH 8.0) and then twice with chloroform alone. The DNA was precipitated in 0.1 volumes of 3 M sodium acetate (pH 5.2) and 2.5 volumes of 100% ethanol and resuspended in 10 mM Tris and 1 mM EDTA (pH 8.0).

The DNA content of samples was estimated using a spectrophotometer (UV-1200, Shimadzu Corp., Kyoto, Japan). The samples were then stored at -20°C pending analysis.

POLYMERASE CHAIN REACTION

Chromosome 10 polymorphisms were detected by PCR using a total of eight microsatellite markers (D10S249, D10S191, D10S210, D10S219, D10S246, D10S222, D10S221, and D10S212). The primers used

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were as suggested by Weissenbach *et al.*¹⁶ and were purchased from Research Genetics Inc., Huntsville, Alabama, USA.

Each PCR volume (20 µl) contained 200 ng genomic DNA, 0.5 mM primer, 200 mM deoxynucleotide triphosphate, and 1 unit of Taq polymerase in a buffer containing 2.5 mM Tris-HCl (pH 8.3), 50 mM KCl and 1.5 mM MgCl₂ (TaKaRa Syuzo Corp., Shiga, Japan). PCR conditions were as follows: 94°C for one minute, 53°C for two minutes and 72°C for one minute in an automated DNA thermal cycler (Sanyo Electric Corp., Osaka, Japan) for 35 cycles.

ELECTROPHORESIS AND STAINING

Ten microlitres of each PCR product were loaded onto a polyacrylamide gel cassette (Multi Gel 15/25, Daiichi Pure Chemicals, Tokyo, Japan) and subjected to electrophoresis for one hour at 40 mA constant current. The gels were stained with 0.5 µg/ml ethidium bromide and photographed using an ultraviolet transilluminator (Spectroline, TM-312A, Spectronics, Westbury, New York, USA).

Results

Figure 1 shows typical LOH at the D10S249, D10S222 and D10S221 loci in case 9. Results at initial surgery are summarised in fig 2. Neither LOH nor homozygous deletion was observed in any of the cases of astrocytoma. Five (55.6%) of nine cases of anaplastic astrocytoma presented with LOH at one to three loci: three of seven informative cases at D10S249; one of eight informative cases at D10S191; two of nine informative cases at D10S246; one of nine informative cases at D10S221; and one of nine informative cases at

D10S212. LOH was observed in all five cases of glioblastoma: one of four informative cases at D10S249; two of five informative cases at D10S210; three of five informative cases at D10S219; one of five informative cases at D10S246; two of four informative cases at D10S222; one of four informative cases at D10S221; and one of five informative cases at D10S212.

Figure 3 summarises the results of SDS-PAGE analysis of all recurrent cases. Tumours in cases 1 and 2, originally classified as astrocytoma, were re-classified as anaplastic astrocytoma at recurrence. Neither LOH nor homozygous deletion was found in these tumours. Four of nine cases were diagnosed as anaplastic astrocytoma both initially and at recurrence (cases 3–6). LOH was not detected in these tumours on either occasion. The remaining five cases of anaplastic astrocytoma were re-classified as glioblastoma at recurrence. Additional LOH was detected in these cases at recurrence (cases 7–11). Four of the five cases initially diagnosed as glioblastoma (cases 12–16) developed additional LOH at recurrence (cases 12–15). Additional LOH observed in these nine cases (cases 7–15) was frequently located at the D10S222 and D10S221 loci (five of eight informative cases for each locus). Additional LOH was observed less frequently at D10S219 (three of nine informative cases), D10S191 (two of eight informative cases), D10S246 (two of nine informative cases), D10S249 and D10S210 (one of eight informative cases for each locus), and D10S212 (one of nine informative cases). Interestingly, of the five cases of anaplastic astrocytoma which progressed to glioblastoma, accumulation of new LOH occurred particularly at D10S222 (four instances; cases 7–10) and D10S221 (three instances; cases 7–9).

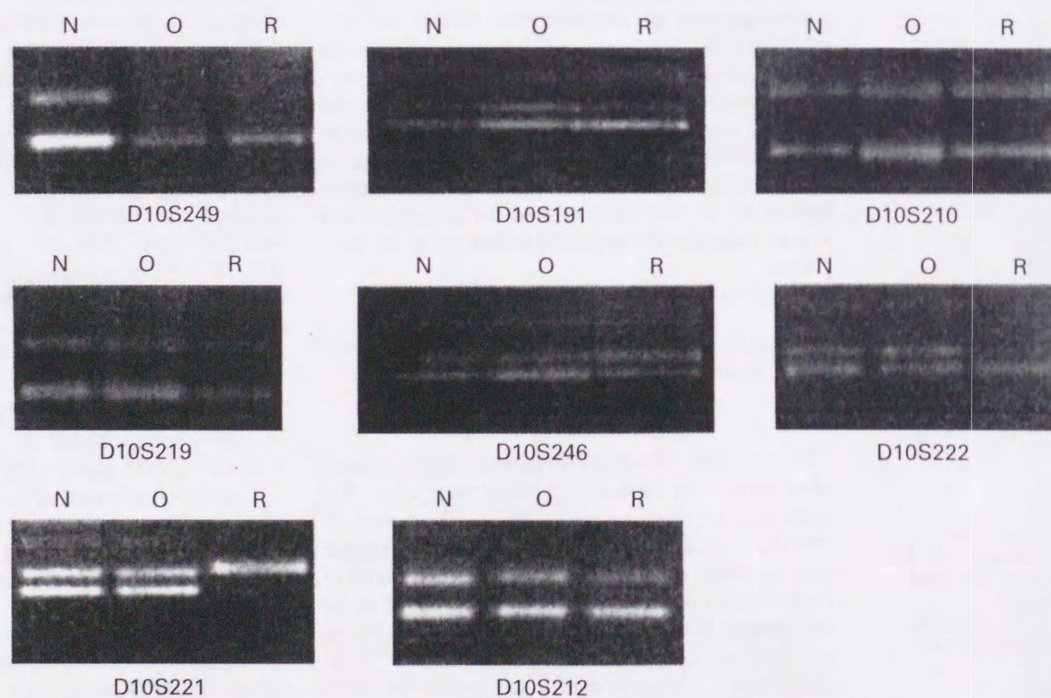


Figure 1 An example of PCR analysis of LOH on chromosome 10 in normal brain tissue (N), original tumour (O) and recurrent tumour (R) in case 9. LOH was detected at D10S249 in the original tumour and at D10S249, D10S222 and D10S221 in the recurrent tumour.

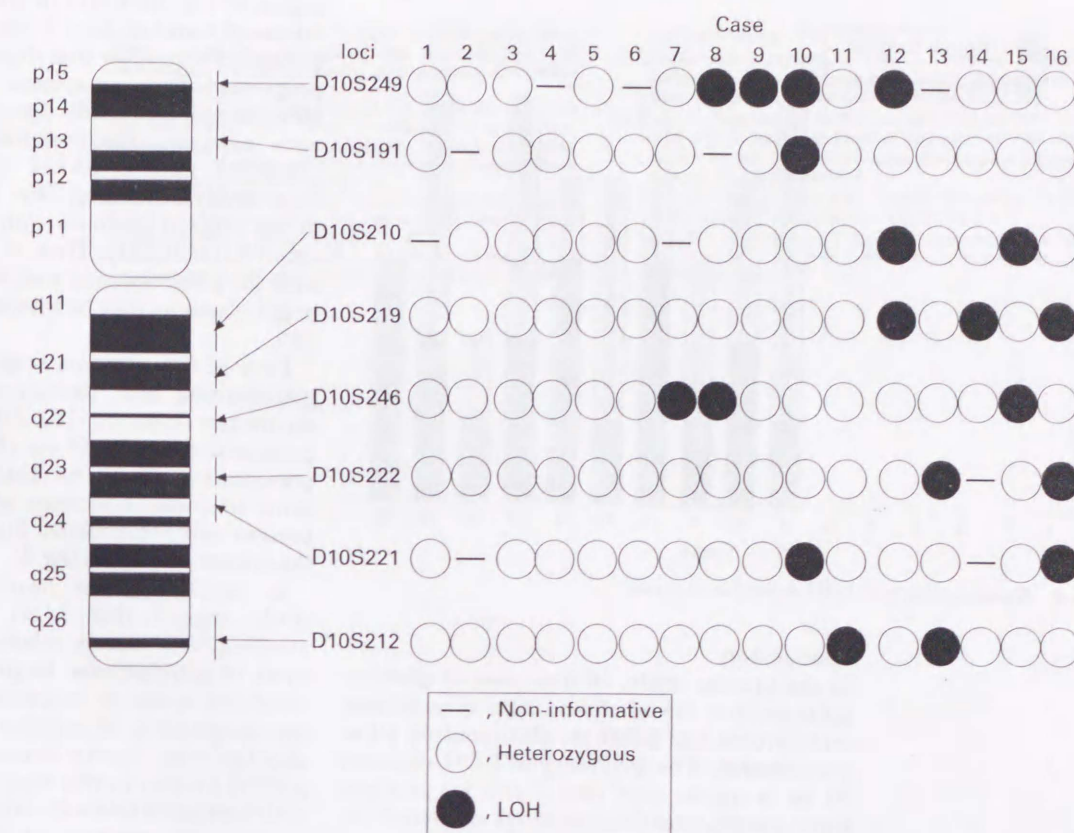


Figure 2 Summary of PCR analysis of the 16 original tumours. Cases 1 and 2 are astrocytomas, cases 3–11 are anaplastic astrocytomas, and cases 12–16 are glioblastomas.

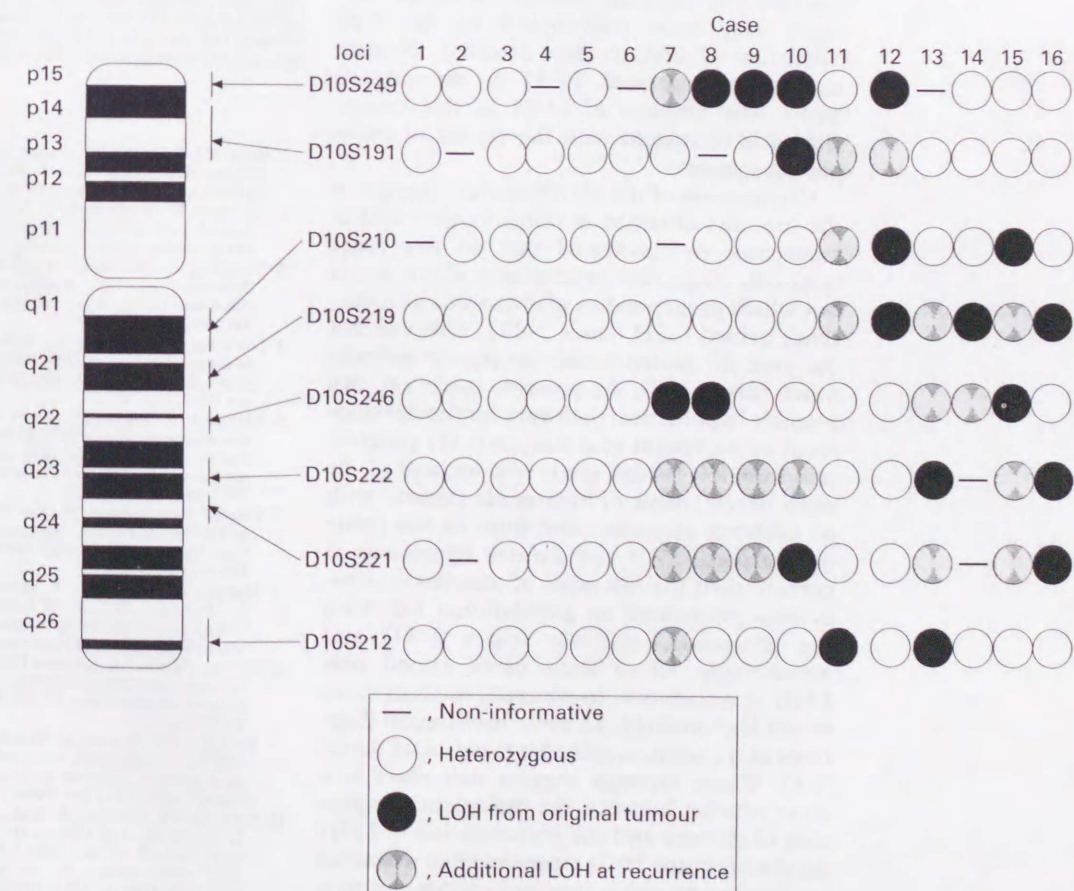


Figure 3 Summary of PCR analysis of the 16 recurrent tumours. Cases 1–6 were diagnosed as anaplastic astrocytomas, and cases 7–16 as glioblastomas at recurrence.

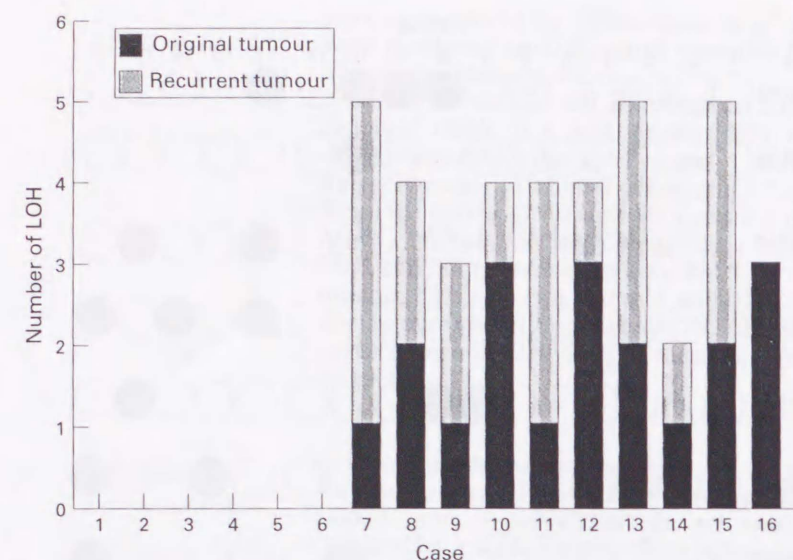


Figure 4 Number of loci with LOH detected in each case.

Discussion

In the present study, all five cases of glioblastoma and five (56%) of nine cases of anaplastic astrocytoma had LOH on chromosome 10 at presentation. The frequency of LOH detected by us is higher than that found by previous investigators, who demonstrated an overall frequency of LOH of 60–95% in glioblastoma and of 12–15% in anaplastic astrocytoma.^{1 4 11 12 17–19} Separation of the tumour tissue from the surrounding brain tissue and the use of multiple microsatellite markers in the present study may have contributed to the high frequency of abnormality detected. Neither astrocytomas showed LOH in the present study. The absence of LOH in this benign glioma is consistent with the results of many previous reports.^{1 4 11 12 17–19}

Comparison of the chromosomal changes in the tumours obtained at initial surgery and at recurrence demonstrated that all five cases originally diagnosed as anaplastic astrocytoma but which progressed to glioblastoma at recurrence gained LOH (cases 7–11). This was not the case for patients with anaplastic astrocytoma (cases 3–6). At present, there are two possible hypothetical pathways for the development of malignant glial tumours: (1) progression from low to high grade tumour; and (2) de novo development of high grade tumour with no evidence of progression from its low grade counterpart.^{11 18} If the former hypothesis is correct, then the five cases of anaplastic astrocytoma progressed to glioblastoma following the progression pathway (cases 7–11) and interestingly, all of these cases gained new LOH at recurrence. In contrast, the four cases which had retained the same histological diagnosis at recurrence, gained no new LOH (cases 3–6). These findings suggest that there is a close relation between the malignant progression of gliomas and the accumulation of LOH on chromosome 10. It is possible that a tumour suppressor gene(s) is localised on this chromosome. Previous deletion mapping studies in glial tumours suggested three candidate loci for the tumour suppressor genes: at the telomeric

region of the short arm of chromosome 10, at 10cen-q23 and at 10q23-qter.^{13 14 17} The data presented here show that those tumours which progressed from anaplastic astrocytoma to glioblastoma frequently demonstrated LOH at two adjacent microsatellite marker loci, D10S222 and D10S221 (80% and 75%, respectively). None of these loci were deleted in the original tumours with the exception of case 10 (D10S221). Thus, the gene(s) responsible for progression of anaplastic astrocytoma to glioblastoma may be located close to 10q23-q25.

Four of five tumours originally classified as glioblastoma also developed new LOH at recurrence (cases 12–15). All of these tumours possessed some LOH on chromosome 10 at presentation. Thus, the instability of chromosome 10 seems to increase when some LOH is present and accumulates further LOH during the course of disease (fig 4).

In conclusion, the results of the present study suggest that LOH on chromosome 10q23-q25 is closely related to the development of glioblastoma. Regional chromosomal instability seems to be especially important in the progression of anaplastic astrocytoma to glioblastoma. Further studies to identify the gene(s) located in this region and to elucidate their biological role will contribute significantly to our understanding of the oncogenesis of human gliomas.

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Figure 1: Types of violence against women

...the most common type of violence against women is physical violence, followed by sexual violence, emotional violence, financial violence, and stalking. The percentage of women who reported physical violence was 75%, sexual violence was 65%, emotional violence was 85%, financial violence was 45%, and stalking was 55%.

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