

# Loss of Heterozygosity at 11p15 in Malignant Glioma<sup>1</sup>

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## ABSTRACT

Deletions of loci on chromosome 11p have been found frequently in several malignant tumors including gliomas, suggesting the presence of tumor suppressor genes. We analyzed 38 gliomas [26 malignant gliomas (grades III and IV) and 12 less malignant gliomas (grades I and II)] for loss of heterozygosity using microsatellite sequences on 11p as polymorphic markers. Loss of heterozygosity was found in 8 of 26 malignant gliomas (31%) but not in the less malignant gliomas. In the region with loss of heterozygosity, loci on 11p15.5-pter were commonly deleted. Our results suggest that a putative tumor suppressor gene involved in malignant progression of gliomas is located in an approximately 21-cM region on 11p15.5-pter.

## INTRODUCTION

Gliomas are the most common malignant disorders of the central nervous system and are divided into four stages according to the WHO classification scheme (1). Less malignant gliomas are defined as grades I and II, while grade III and IV gliomas are malignant. Cytogenetic and molecular genetic studies have drawn attention to the loss of specific chromosomal regions in gliomas, suggesting the presence of tumor suppressor genes. Studies of LOH<sup>3</sup> in gliomas have suggested the presence of tumor suppressor genes on chromosomes 9p, 10p, 10q, 11p, 13q, 17p, 19q, and 22q (2–4). However, only the *p53* gene on 17p (5) and the *MTS1/p16* and *MTS2/p15* genes on 9p (6, 7) have been identified as the responsible genes. To isolate putative glioma suppressor genes on the remaining chromosomes, narrowing down of a commonly deleted region has been attempted (8).

Regions on 11p are of particular interest since 11p loci are frequently deleted in several cancers, including glioma (9–13). In this study, we constructed a deletion map of 11p in glioma using 18 polymorphic markers.

## MATERIALS AND METHODS

**Human Tissue Samples.** All glioma samples were obtained surgically. The tumors were classified according to the WHO brain tumor scheme. Gliomas examined in this study consisted of one ependymoma (grade I), 10 astrocytomas (grade II), 2 oligodendrogliomas (grade II), 12 anaplastic astrocytomas (grade III), 3 anaplastic ependymomas (grade III), and 11 glioblastomas (grade IV). Resected tumors were immediately frozen in liquid nitrogen and stored at  $-70^{\circ}\text{C}$  until analysis. Control DNA for each tumor was obtained from leukocytes isolated from frozen peripheral blood or from normal brain tissue of the same individual.

**PCR Analysis of Microsatellite Markers.** The polymorphic markers examined are listed in Table 1. Primers with which to amplify marker DNAs were synthesized according to the reported nucleotide sequences (14–20). For the *D11S988* locus, the nucleotide sequence was obtained from a database

(GDB No. G00-195-012). PCR was performed using 50 ng of template DNA in a volume of 5  $\mu\text{l}$  with 30 cycles of  $94^{\circ}\text{C}$  for 20 s and  $60^{\circ}\text{C}$  for 2 min in a Gene Amp PCR system 9600 (Perkin-Elmer Cetus). The reaction mixture was diluted 5–50-fold with 95% formamide-20 mM EDTA-0.05% xylene cyanol-0.05% bromophenol blue and then denatured at  $80^{\circ}\text{C}$  for 3 min. Electrophoresis proceeded in 5% polyacrylamide gels containing 7 M urea.

**PCR-Single-Strand Conformation Polymorphism Analysis.** The polymorphic sequence of the *Apal* recognition site (GGACCC/GGGCCC) (14) at the *IGF2* locus was analyzed by means of PCR-single-strand conformation polymorphism (21). Electrophoresis in 5% polyacrylamide containing 5% glycerol proceeded at 40 W at  $10^{\circ}\text{C}$ .

## RESULTS

The frequency of LOH detected by analysis of polymorphic markers is summarized in Table 1. Nine of 26 malignant gliomas (35%) showed LOH at least one locus on chromosome 11, but none of the less malignant types had LOH at any chromosome 11 locus examined.

Representative results of the LOH analysis are shown in Fig. 1. For example, a comparison of cancerous and normal DNA from patient 13 revealed LOH at the *D11S1318*, *D11S988*, *D11S1245*, and *D11S1256* loci. Although the signal for the *D11S922* locus of this patient was not informative, the results together with those of other loci indicated the total loss of chromosome 11. LOHs at loci on chromosome 11 in 9 malignant gliomas including tumor 13 are summarized in Fig. 2. The regions on the short arm were lost in these tumors. The only exception was tumor 20, which had LOH at the locus on 11q23, but it retained heterozygosity at all loci on 11p analyzed. Tumor 25 showed LOH at all informative loci on 11p but retained heterozygosity at loci on 11q, indicating the loss of an entire short arm. In tumors 1, 8, 15, 18, 23, and 24, LOH was found only in the telomeric portion of 11p. In the eight tumors with LOH at the 11p loci, the region of 11p15 indicated by two arrows was commonly deleted. Based on the information available to the markers, the region commonly deleted from the *D11S988* locus to the pter could be about 21 cM (22, 23).

Microsatellite instability was not detected at any loci on chromosome 11 examined.

## DISCUSSION

In glioma, the most frequently reported genetic alterations are mutations or deletions of the *p53*, *p16/MTS1*, and *p15/MTS2* genes, LOH at chromosomes 17p and 10; and amplification and rearrangement of the epidermal growth factor receptor (*EGFR*) gene (2, 3).

The *p53* gene is mutated in gliomas of all grades of malignancy (2). Thus, alterations of the *p53* gene may play a role in early glioma formation.

LOH on chromosome 10 has not been found in gliomas with a low grade of malignancy. On the other hand, allelic losses on chromosome 10 have been detected frequently in malignant gliomas, especially in grade IV tumors, suggesting that putative tumor suppressor genes on the chromosome are involved in progression of tumors to grade IV. The amplification and rearrangement of the *EGFR* gene in glioblastomas might be aberrations in a late stage of tumorigenesis, because these were found in tumors with LOH on chromosome 10. Other genetic changes, such as deletions of regions on chromosomes 9p

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<sup>3</sup> The abbreviation used is: LOH, loss of heterozygosity.

(*p16/MTS1* and *p15/MTS2*), 11p, 13q, 19q, and 22, may also play a role in the formation of some portion of glioblastomas (3, 4, 8, 10).

In this study, we detected a frequent LOH at loci on chromosome 11p only in malignant gliomas. There was no difference in the frequency in gliomas between grades III and IV. This suggests that the putative tumor suppressor gene on chromosome 11p plays an important role in the progression of gliomas to grade III.

Fults *et al.* (10) have found LOH in 26% of malignant astrocytomas and in 27% of primitive neuroectodermal tumors and suggested that a region on 11p14–pter was commonly deleted. Our results indicated that the region commonly deleted in malignant gliomas is distal to the marker *D11S988*. This region overlaps that reported, and we narrowed it down to about 21 cM. These results indicated that a putative glioma tumor suppressor gene(s) is located on 11p15.5–pter. Loci are fre-

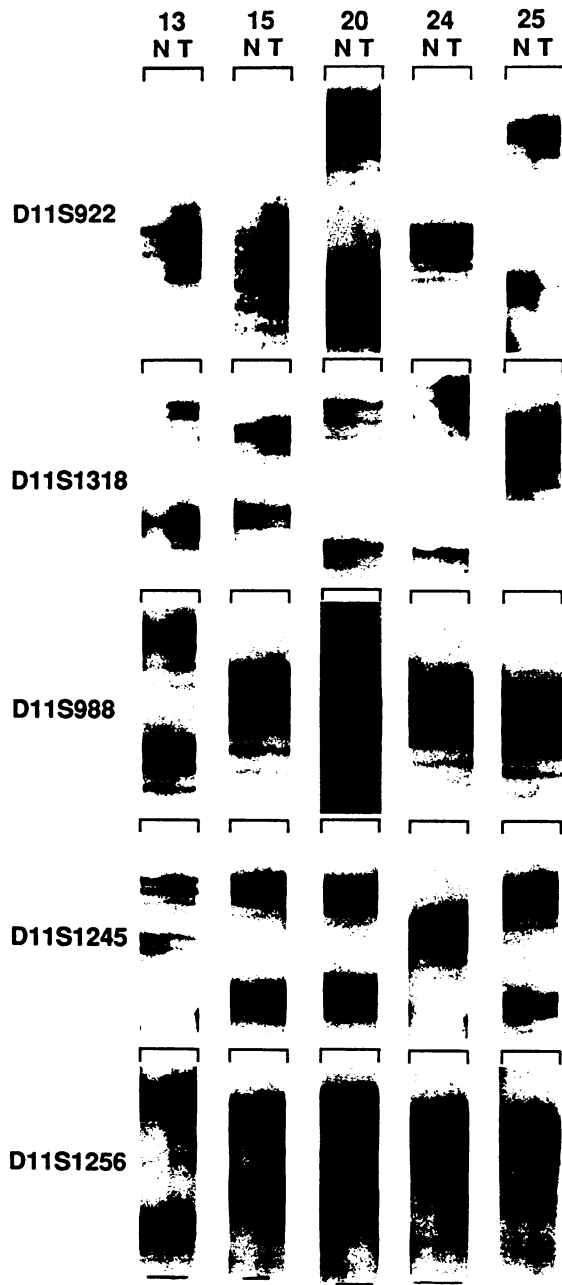


Fig. 1. LOH at loci on chromosome 11 in malignant gliomas. *N* and *T*, normal and tumor DNA from individual patients, respectively. LOH in tumor samples was detected by a loss of the signal intensity of polymorphic markers amplified from tumor DNA compared with those from normal DNA.

Table 1 LOH at loci on chromosome 11 in human gliomas

Locus symbol	Location	Repeating sequence	Frequency of LOH	
			Less malignant glioma (grade I and II)	Malignant glioma (grade III and IV)
<i>HRAS</i>	11p15.5	GGGCCT	0/3 (0) <sup>a</sup>	2/11 (18)
<i>D11S1363</i>	11p15.5	CA	0/4 (0)	5/9 (56)
<i>D11S922</i>	11p15.5	CA	0/6 (0)	7/24 (29)
<i>IGF2</i>	11p15.5	<sup>b</sup>	0/6 (0)	4/15 (27)
<i>TH</i>	11p15.5	TCAT	0/7 (0)	4/20 (20)
<i>D11S1318</i>	11p15.5	CA	0/8 (0)	6/20 (30)
<i>D11S860</i>	11p15.4–5	CA	0/9 (0)	5/21 (24)
<i>D11S988</i>	11p15.4	CA	0/3 (0)	3/10 (30)
<i>HBB</i>	11p15.4	CA	0/7 (0)	5/20 (25)
<i>D11S1250</i>	11p15.2–3	CA	0/10 (0)	5/22 (23)
<i>D11S1245</i>	11p15.1	CA	0/8 (0)	3/22 (14)
<i>D11S1246</i>	11p14	CA	0/8 (0)	2/19 (11)
<i>D11S1252</i>	11p11.2–12	CA	0/13 (0)	2/23 (9)
<i>D11S1261</i>	11q13.4–21	CA	0/5 (0)	0/15 (0)
<i>D11S938</i>	11q23	CA	Not done	1/14 (7)
<i>D11S1256</i>	11q23	CA	0/13 (0)	2/24 (8)
<i>D11S939</i>	11q23	CA	Not done	1/14 (7)
<i>D11S1263</i>	11q23.2–24	CA	0/10 (0)	1/20 (5)

<sup>a</sup> Numbers in parentheses, percentage.

<sup>b</sup> A polymorphic single-base substitution was detected by single-strand conformation polymorphism analysis.

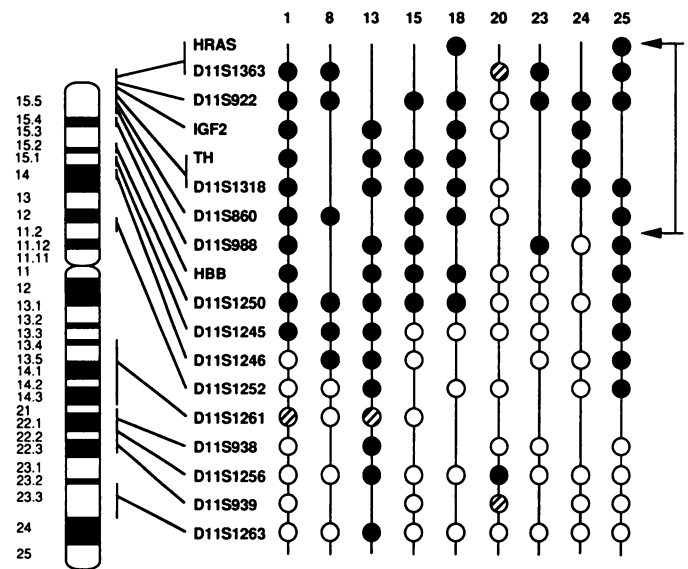


Fig. 2. Allelic losses in 9 patients with LOH at loci on chromosome 11. *Top abscissa*, tumor numbers; ● and ○, loss and retention of heterozygosity, respectively; ⊗, samples in which the results are inconclusive or not interpretable; *Vertical lines*, noninformative samples. The region commonly deleted is indicated by a vertical bar with arrows.

quently deleted from 11p15 in other human neoplasias (9), suggesting that a tumor suppressor gene(s) is involved in many types of cancer.

Tumors 13 and 20 showed LOH at 11q23. To our knowledge, LOH at 11q has not been found in gliomas. In tumor 20, a limited region of 11q was deleted, whereas tumor 13 has completely lost chromosome 11. Recently, deletions of loci on 11q22–24 have been found in other cancers such as breast and colorectal carcinomas (24, 25). These findings suggest the presence of a tumor suppressor gene in this region.

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REFERENCES

1. Zülch, K. J. Histological typing of tumours of the central nervous system. Geneva: WHO, 1979.

2. Lang, F. F., Miller, D. C., Koslow, M., and Newcomb, E. W. Pathways leading to glioblastoma multiforme: a molecular analysis of genetic alterations in 65 astrocytic tumors. *J. Neurosurg.*, *81*: 427–436, 1994.
3. Fults, D., Pedone, C. A., Thomas, G. A., and White, R. Allelotype of human malignant astrocytoma. *Cancer Res.*, *50*: 5784–5789, 1990.
4. Rey, J. A., Bello, M. J., de Campos, J. M., Vaquero, J., Kusak, M. E., Sarasa, J. L., and Pestaña, A. Abnormalities of chromosome 22 in human brain tumors determined by combined cytogenetic and molecular genetic approaches. *Cancer Genet. Cytogenet.*, *66*: 1–10, 1993.
5. Mashiyama, S., Murakami, Y., Yoshimoto, T., Sekiya, T., and Hayashi, K. Detection of *p53* gene mutations in human brain tumors by single-strand conformation polymorphism analysis of polymerase chain reaction products. *Oncogene*, *6*: 1313–1318, 1991.
6. Kamb, A., Gruis, N. A., Weaver-Feldhaus, J., Liu, Q., Harshman, K., Tavtigian, S. V., Stockert, E., Day, R. S., III, Johnson, B. E., and Skolnick, M. H. A cell cycle regulator potentially involved in genesis of many tumor types. *Science (Washington DC)*, *264*: 436–440, 1994.
7. Jen, J., Harper, J. W., Bigner, S. H., Binger, D. D., Papadopoulos, N., Markowitz, S., Willson, V. K. J., Kinzler, K. W., and Vogelstein, B. Deletion of *p16* and *p15* genes in brain tumors. *Cancer Res.*, *54*: 6353–6358, 1994.
8. Rubio, M., Correa, K. M., Ueki, K., Mohrenweiser, H. W., Gusella, J. F., von Deimling, A., and Louis, D. N. The putative glioma tumor suppressor gene on chromosome 19q maps between *APOC2* and *HRC*. *Cancer Res.*, *54*: 4760–4763, 1994.
9. Seizinger, B. R., Klinger, H. P., Junien, C., Nakamura, Y., Le Beau, M., Cavenee, W., Emanuel, B., Ponder, B., Naylor, S., Mitelman, F., Louis, D., Menon, A., Newsham, I., Decker, J., Kaelbling, M., Henry, I., and von Deimling, A. Report of the committee on chromosome and gene loss in human neoplasia. *Cytogenet. Cell Genet.*, *58*: 1080–1096, 1991.
10. Fults, D., Petronio, J., Noblett, B. D., and Pedone, C. A. Chromosome 11p15 deletions in human malignant astrocytomas and primitive neuroectodermal tumors. *Genomics*, *14*: 799–801, 1992.
11. Ali, U. I., Lidereau, R., Theillet, C., and Callahan, R. Reduction to homozygosity of genes on chromosome 11 in human breast neoplasia. *Science (Washington DC)*, *238*: 185–188, 1987.
12. Bepler, G., and Garcia-Blanco, M. A. Three tumor-suppressor regions on chromosome 11p identified by high-resolution deletion mapping in human non-small-cell lung cancer. *Proc. Natl. Acad. Sci. USA*, *91*: 5513–5517, 1994.
13. Winqvist, R., Mannermaa, A., Alavaikko, M., Blanco, G., Taskinen, P. J., Kiviniemi, H., Newsham, I., and Cavenee, W. Refinement of regional loss of heterozygosity for chromosome 11p15.5 in human breast tumors. *Cancer Res.*, *53*: 4486–4488, 1993.
14. Tadokoro, K., Fujii, H., Inoue, T., and Yamada, M. Polymerase chain reaction (PCR) for detection of *Apal* polymorphism at the insulin like growth factor II gene (*IGF2*). *Nucleic Acids Res.*, *19*: 6967, 1991.
15. Tanci, P., Genuardi, M., Santini, S. A., and Neri, G. PCR detection of an insertion/deletion polymorphism in intron 1 of the *HRAS1* locus. *Nucleic Acids Res.*, *20*: 1157, 1992.
16. Gyapay, G., Morissette, J., Vignal, A., Dib, C., Fizames, C., Millasseau, P., Marc, S., Bernardi, G., Lathrop, M., and Weissenbach, J. The 1993–94 Généthon human genetic linkage map. *Nat. Genet.*, *7*: 246–339, 1994.
17. Polymeropoulos, M. H., Xiao, H., Rath, D. S., and Merrill, C. R. Tetranucleotide repeat polymorphism at the human tyrosine hydroxylase gene (*TH*). *Nucleic Acids Res.*, *19*: 3753, 1991.
18. McNoe, L. A., Eccles, M. R., and Reeve, A. E. Dinucleotide repeat polymorphism at the *D11S860* locus. *Nucleic Acids Res.*, *20*: 1161, 1992.
19. Iizuka, M., Jones, C., Hayashi, K., and Sekiya, T. Mapping of 28 (CA)<sub>n</sub> microsatellite repeats and 13 *Alu* markers on human chromosome 11 using a panel of somatic cell hybrids. *Genomics*, *19*: 581–584, 1994.
20. Hauge, X. Y., Litt, R., and Litt, M. Dinucleotide repeat polymorphisms at the *D11S439* and *HBB* loci. *Hum. Mol. Genet.*, *1*: 548, 1991.
21. Orita, M., Suzuki, Y., Sekiya, T., and Hayashi, K. Rapid and sensitive detection of point mutations and DNA polymorphisms using polymerase chain reaction. *Genomics*, *5*: 874–879, 1989.
22. Kramer, P., Becker, W., Heutink, P., James, M., Julier, C., Lathrop, M., Luty, J. A., Wang, Z., Weber, J. L., Wilkie, P., and Litt, M. Chromosome 11. *Science (Washington DC)*, *258*: 67–86, 1992.
23. James, M. R., Richard, C. W., III, Schott, J.-J., Yousry, C., Clark, K., Bell, J., Terwilliger, J. D., Hazen, J., Dubay, C., Vignal, A., Agrapart, M., Imai, T., Nakamura, Y., Polymeropoulos, M., Weissenbach, J., Cox, D. R., and Lathrop, G. M. A radiation hybrid map of 506 STS markers spanning human chromosome 11. *Nat. Genet.*, *8*: 70–76, 1994.
24. Carter, S. L., Negrini, M., Baffa, R., Gillum D. R., Rosenberg, A. L., Schwartz, G. F., and Croce, C. M. Loss of heterozygosity at 11q22–q23 in breast cancer. *Cancer Res.*, *54*: 6270–6274, 1994.
25. Keldysh, P. L., Dragani, T. A., Fleischman, E. W., Konstantinove, L. N., Perevoschikov, A. G., Pierotti, M. A., Della Porta, G., and Kopnin, B. P. 11q deletions in human colorectal carcinomas: cytogenetic and restriction fragment length polymorphism analysis. *Genes Chromosomes Cancer*, *6*: 45–50, 1993.

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