TABLE 3

*Review of the literature in patients with GBM who survived longer than 5 years**

Authors & Year	No. of Cases	Mean Age (yrs)	M/F Ratio	Mean KPS Score	No. of Patients w/ (%)				OS (no. of patients)	
					GTR	RT	Chemo	GCGBMs	<10 Yrs	>10 Yrs
Netsky, et al., 1950	5	24–42†	1:1‡	NA	NA	2‡	NA	NA	4	1
Bouchard & Peirce, 1960	9	NA	NA	NA	NA	9	0	NA	4	5
Roth & Elvidge, 1960	12	NA	4:8	NA	7	8	0	NA	9	3
Ley, et al., 1962	3	NA	NA	NA	NA	3	0	NA	3	0
Taveras, et al., 1962	7	NA	NA	NA	0	7	0	NA	7	0
Elvidge & Barone, 1965	2§	24.5‖	1:1	NA	2	1	0	1	0	2
Gullotta & Bettag, 1967	1	45	1:0	NA	1	1	0	NA	0	1
Jelsma & Bucy, 1967	4	39‖	3:0‡	NA	4	3‡	0	NA	4	0
Takeuchi, 1975	2	38.5‖	2:0	NA	NA	2	2	NA	2	0
Dara, et al., 1980	1	32	NA	NA	NA	1	1	NA	1	0
Johnson, 1981	1	32	0:1	NA	1	0	0	1: PXA?	0	1
Hatanaka, et al., 1984	1	50	1:0	100	0	1	0	NA	0	1
Bucy, et al., 1985	1	30	1:0	NA	1	1	0	1: AOA?	0	1
Salford, et al., 1988	2	14.5‖	1:1	NA	1	2	0	1**	0	2
Akslen, et al., 1989	2	41.5‖	1:1	NA	2	2	1	2	2	0
Ishikura, et al., 1989	1	8	0:1	NA	1	0	0	1	1	0
Margetts & Kalyan-Raman, 1989	1	41	1:0	NA	1	1	1	1	1	0
Imperato, et al., 1990	5	42.6‖	3:2	NA	5	5	4	NA	3	2
Shibamoto, et al., 1990	1	51	1:0	NA	1	1	0	NA	1	0
Rutz, et al., 1991	1	21	1:0	NA	1	1	0	NA	0	1
Vertosick & Selker, 1992	10	39.9‖	6:4	86‖	NA	10	10	NA	6	4
Chandler, et al., 1993	22	39.2	10:12	80	2	22	18	NA	17	5
Hiesiger, et al., 1993	4	41.5‖	NA	92.5‖††	0	4	4	NA	4	0
Phuphanich, et al., 1993	1	33	1:0	90	NA	1	1	1	1	0
Archibald, et al., 1994	7	37.7‖	2:5	NA	2	7	7	NA	7	0
Wester, et al., 1994	1	45	0:1	NA	1	1	0	0	1	0
Morita, et al., 1996	10	39.2‖	7:3	NA	10	NA	NA	NA	8	2
New, et al., 1997	1	34	0:1	100	1	1	1	NA	1	0
Pollak, et al., 1997	2	20‖	1:1	NA	2	2	0	0	0	2
Cervoni, et al., 1998	1	13	0:1	80	1	1	1	1	0	1
Klein, et al., 1998	1	11	0:1	NA††	1	1	1	1	0	1
Puzzilli, et al., 1998	1	50	1:0	90	0	1	0	1	0	1
Salvati, et al., 1998	11	39	5:6	80	11	11	11	NA	10	1
Scott, et al., 1999	7	46.9‖	4:3	88.6‖	NA	7	NA	NA	4	3
Yoshida, et al., 2000	2	40‖	1:1	NA	1	2	2	2	0	2
Sabel, et al., 2001	1	69	1:0	NA	1	0	0	1	0	1
present study	6	44.2	0:6	85	4	6	6	3	6	0

* AOA = anaplastic oligoastrocytoma; chemo = chemotherapy; NA = not available due to insufficient data; PXA = pleomorphic xanthoastrocytoma; RT = radiation therapy; ? = possibly.
† Only the age range was available.
‡ Data were only available for some patients.
§ Data on these patients were included in the report by Roth and Elvidge.
‖ Specific data not given in original publication, but were calculated on the basis of data that were provided.
** Patient underwent PR.
†† Postoperative KPS score.

recurrence. The patient suffered a gradual disturbance in consciousness that was attributable to radiation-induced, progressive diffuse brain atrophy (Fig. 1C) and died of respiratory complications approximately 6.5 years after establishment of the diagnosis.

Case 4

This 31-year-old woman experienced a progressively worsening headache for approximately 1 month. Magnetic resonance images demonstrated a ringlike enhanced mass lesion in the left frontal lobe (Fig. 1D). Macroscopically, the tumor appeared to be better demarcated than is typical of GBM, and the patient underwent gross-total resection in May 1995 (Fig. 1E). A histopathological evaluation returned a diagnosis of giant cell GBM (Fig. 2C and D). During a 1-year period, the woman received radiation therapy in conjunction with five courses of IV-ACNU (total 540 mg). She was able to resume her normal life and had a KPS score of 100. Seven and one-half years after the initial diagnosis, an irregular contrast-enhanced lesion was demonstrated by MR imaging in the contralateral frontal lobe (Fig. 1F). Although biopsy confirmed tumor recurrence, at the time of this writing the patient remains alive but bedridden.

Case 6

This 60-year-old woman was involved in a traffic accident caused by an epileptic seizure she experienced in May 1998. Computerized tomography scans demonstrated a ringlike enhanced mass lesion in her left frontal lobe (Fig. 1G). Intraoperatively the tumor appeared well demarcated

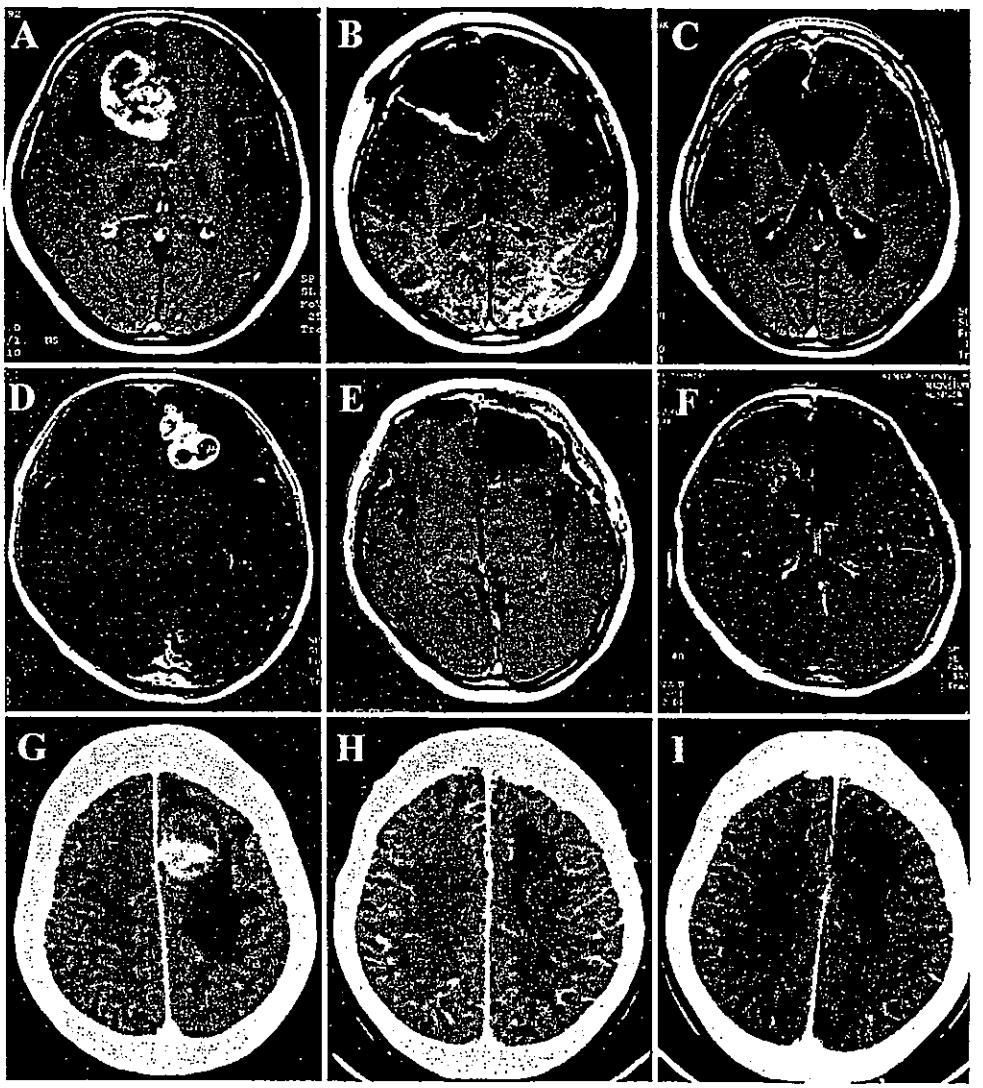


FIG. 1. Contrast-enhanced CT and MR images. A–C: Case 3. Note the irregularly shaped ringlike enhanced mass lesion with peritumoral edema on this preoperative image (A). Following gross-total resection an area of hyperintensity at the bottom of the resection cavity is a local postoperative change (B). Six years after gross-total resection there is a marked brain cortical atrophy (C). D–F: Case 4. The left frontal tumor (D) was totally resected (E). Enhanced lesions in the right frontal lobe were visualized 7.5 years later (F) and tumor recurrence was confirmed by biopsy. G–I: Case 6. The left frontal tumor (G) was totally removed (H), and there was no sign of tumor recurrence 5 years later (I).

and a gross-total resection was performed (Fig. 1H). A histopathological diagnosis of giant cell GBM was returned (Fig. 2E and F). The patient received radiation therapy in conjunction with PAV-I; procarbazine (total 90 mg), ACNU (total 105 mg), vincristine (total 4 mg), and interferon-β (3×10^6 U three times per week) were administered in a one 6-week course. At the time of this writing she is able to pursue her normal life, her KPS score is 100, and there has been no tumor recurrence (Fig. 1I).

Discussion

Glioblastoma multiforme has remained incurable despite the use of multimodal aggressive treatments; the median duration of survival among patients with GBM is approximately 1 year[13,18,24,36,53,60] and only 1 to 5% of patients with this disease survive for more than 3 to 5 years.[11,42,46,47,49,50,55,58] The long-term survival rate in patients treated at our institution and assessed in this study was 5.3%, similar to the reported rate.

Reported positive prognostic factors associated with long-term survival are a relatively young age, a higher preoperative KPS score, aggressive resection, radiotherapy, adjuvant chemotherapies, and a prolonged PFS period.[10,11,33,42,45–47,49,50,55,57,58] To identify other potentially favorable prognostic factors in adult patients with supratentorial GBM, we analyzed the clinical data in six long-term survivors selected from a uniform population of Japanese patients enrolled in clinical trials. All had undergone a variety of surgical interventions and subsequent combined radiotherapy and ACNU-based chemotherapy (Table 1). The control

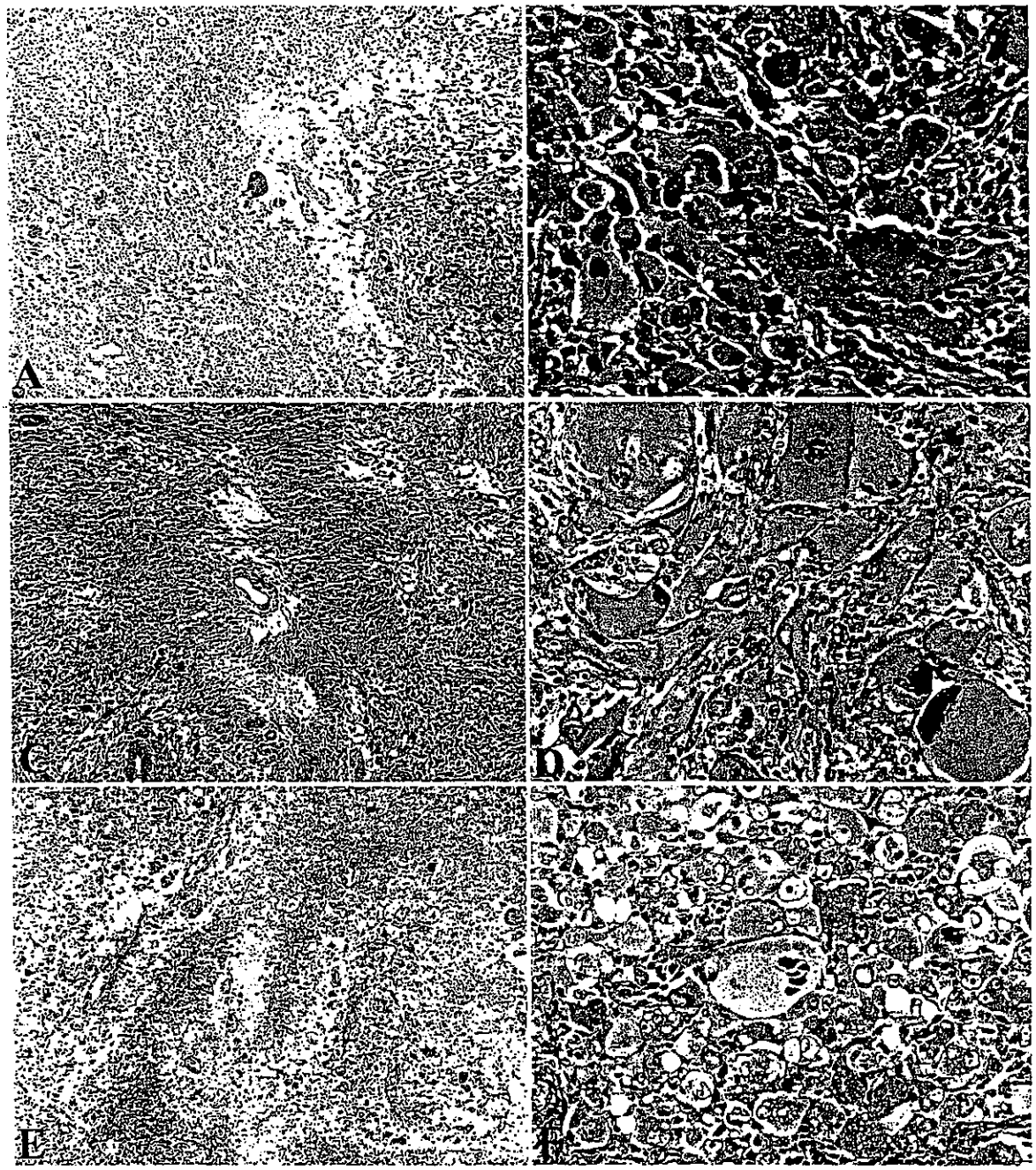


FIG. 2. Photomicrographs showing tumor specimens from Case 3 (A and B), Case 4 (C and D), and Case 6 (E and F). In each case large areas of necrosis and microvascular proliferation (A, C, and E) and marked pleomorphic nuclei (B, D, and F) were identified. In Case 4 there were many areas of pseudopalisading necrosis (C). Numerous giant cells with extremely unusual mono- or multinuclei were found in each case (B, D, and F). H & E, original magnifications × 50 (A, C, and E) and × 200 (B, D, and F).

group was composed of 107 similarly treated patients with GBM who survived for fewer than 5 years (Table 2). As in other studies, we found tendencies among our long-term survivors compared with controls to be younger (their mean age was 44.2 years), have a higher KPS score (the mean score was 85), have undergone an extensive resection, and have a longer PFS period. Besides these known favorable factors, we found that all our six long-term survivors were women and that three of them had histopathologically confirmed giant cell GBM (Table 1). Interestingly, none of the 107 control patients had giant cell GBM (Table 2).

To test our results, we analyzed available data in 150 long-term survivors treated at our institution and at others (Table 3). We found that 61 (50%) of 122 long-term survivors of GBM were female, a rate higher than that reported in patients with GBM who survived for the usual duration of time. There is an acknowledged male predominance among patients with GBM and in our control group the

male/female ratio was 1.5:1 (Table 2). Some studies have already indicated that female patients with GBM have a better prognosis than their male counterparts[8,18,42,48] and that hormones or a tumor suppressor gene(s) on the X chromosome may be associated with their longer survival.[39,51]

It remains controversial whether giant cell GBM is[1,7,27,31,44] or is not[19] associated with better survival among patients with GBM. Although the sample is small, our survey of available data showed that 18 (69.2%) of 26 long-term survivors had a tumor diagnosed as giant cell GBM or one with numerous giant cells. Although giant cell GBM reportedly accounts for approximately 5% of all GBMs,[37] the 69.2% incidence of patients with giant cell GBM or with manifestations of numerous giant cells in our survey of long-term GBM survivors far exceeds the 5% reported rate. In our group of six long-term survivors, all three whose disease was diagnosed as giant cell GBM were treated by gross-total resection. Of the 14 previously reported long-term survivors of giant cell GBM in whom information regarding the surgical procedures was available, 11 (78.6%) underwent gross-total or radical resection. Because giant cell GBMs are macroscopically well-circumscribed tumors, more aggressive resection is possible, resulting in a higher degree of cytoreduction. Therefore, subsequent adjuvant therapy may effectively prolong the postoperative survival in patients with giant cell GBM who undergo radical surgical treatment.

Conclusions

Our investigation shows that, in addition to the known factors associated with long-term survival in adult patients with supratentorial GBM who undergo uniform adjuvant therapy, female sex and histopathological characteristics consistent with giant cell GBM represent favorable prognostic factors.

References

1. Akslen LA, Mork SJ, Larsen JL, et al: Giant cell glioblastoma: a work-up of 2 cases with long survival. **Acta Neurol Scand 79:** 194–199, 1989
2. Albert FK, Forsting M, Sartor K, et al: Early postoperative magnetic resonance imaging after resection of malignant glioma: objective evaluation of residual tumor and its influence on regrowth and prognosis. **Neurosurgery 34:**45–61, 1994
3. Archibald YM, Lunn D, Ruttan LA, et al: Cognitive functioning in long-term survivors of high-grade glioma. **J Neurosurg 80:** 247–253, 1994
4. Bauman GS, Ino Y, Ueki K, et al: Allelic loss of chromosome 1p and radiotherapy plus chemotherapy in patients with oligodendrogliomas. **Int J Radiat Oncol Biol Phys 48:**825–830, 2000
5. Bouchard J, Peirce CB: Radiation therapy in the management of neoplasms of the central nervous system, with a special note in regard to children: Twenty years' experience, 1939–1958. **AJR Rad Ther Nucl Med 84:**610–628, 1960
6. Bucy PC, Oberhill HR, Siqueira EB, et al: Cerebral glioblastomas can be cured! **Neurosurgery 16:**714–717, 1985
7. Burger PC, Vollmer RT: Histologic factors of prognostic significance in the glioblastoma multiforme. **Cancer 46:**1179–1186, 1980
8. Burton EC, Lamborn KR, Forsyth P, et al: Aberrant p53, mdm2, and proliferation differ in glioblastomas from long-term compared with typical survivors. **Clin Cancer Res 8:**180–187, 2002
9. Cairncross JG, Ueki K, Zlatescu MC, et al: Specific genetic predictors of chemotherapeutic response and survival in patients with anaplastic oligodendrogliomas. **J Natl Cancer Inst 90:** 1473–1479, 1998
10. Cervoni L, Celli P, Salvati M: Long-term survival in a patient with supratentorial glioblastoma: clinical considerations. **Ital J Neurol Sci 19:**221–224, 1998
11. Chandler KL, Prados MD, Malec M, et al: Long-term survival in patients with glioblastoma multiforme. **Neurosurgery 32:** 716–720, 1993
12. Dara P, Slater LM, Talreja D, et al: Long-term survivors of high-grade malignant astrocytomas. **Med Pediatr Oncol 8:**187–191, 1980
13. Davis FG, Freels S, Grutsch J, et al: Survival rates in patients with primary malignant brain tumors stratified by patient age and tumor histological type: an analysis based on Surveillance, Epidemiology, and End Results (SEER) data, 1973–1991. **J Neurosurg 88:**1–10, 1998
14. Elvidge AR, Barone BM: Long-term postoperative survival in two cases of glioblastoma multiforme. **J Neurosurg 22:**382–386, 1965
15. Gullotta F, Bettag W: Zür Frage der längeren Überlebenszeit bei Glioblastomen. **Acta Neurochir 16:**122–128, 1967
16. Hatanaka H, Sano K, Kitamura K, et al: CT findings in patients with gliomas surviving more than 10 years. **Neurochirurgia 27:** 106–110, 1984
17. Hiesiger EM, Hayes RL, Pierz DM, et al: Prognostic relevance of epidermal growth factor receptor (EGF-R) and c-neu/erbB2 expression in glioblastomas (GBMs). **J Neurooncol 16:**93–104, 1993
18. Hirano H, Asakura T, Yokoyama S, et al: [The prognostic factors in astrocytic tumors: analysis by the Kaplan– Meier method and the Weibull log-linear model.] **No Shinkei Geka 24:**809–815, 1996 (Jpn)
19. Huang MC, Kubo O, Tajika Y, et al: A clinico-immunohistochemical study of giant cell glioblastoma. **Noshuyo Byori 13:**11–16, 1996
20. Imperato JP, Paleologos NA, Vick NA: Effects of treatment on long-term survivors with malignant astrocytomas. **Ann Neurol 28:**818–822, 1990
21. Ishikura A, Ikeda M, Kogure Y, et al: [A giant cell glioblastoma—a case report.] **Gan No Rinsho 35:**293–297, 1989 (Jpn)
22. Jelsma R, Bucy PC: The treatment of glioblastoma multiforme of the brain. **J Neurosurg 27:**388–400, 1967
23. Johnson AC: Malignant intracranial tumor: report of three cases with long-term postoperative survival. **Surg Neurol 15:**217–219, 1981
24. Kallio M, Sankila R, Jaaskelainen J, et al: A population-based study on the incidence and survival rates of 3857 glioma patients diagnosed from 1953 to 1984. **Cancer 68:**1394–1400, 1991
25. Kleihues P, Burger PC, Scheithauer BW: The new WHO classification of brain tumours. **Brain Pathol 3:**255–268, 1993
26. Kleihues P, Cavenee WK: **Pathology and Genetics of Tumours of the Nervous System. IARC Scientific.** Lyon, France: IARC, 2000
27. Klein R, Molenkamp G, Sorensen N, et al: Favorable outcome of giant cell glioblastoma in a child. Report of an 11-year survival period. **Childs Nerv Syst 14:**288–291, 1998
28. Kochii M, Kitamura I, Goto T, et al: Randomized comparison of intra-arterial versus intravenous infusion of ACNU for newly diagnosed patients with glioblastoma. **J Neurooncol 49:**63–70, 2000
29. Kraus JA, Wenghoefer M, Schmidt MC, et al: Long-term survival of glioblastoma multiforme: importance of histopathological reevaluation. **J Neurol 247:**455–460, 2000
30. Ley A, Ley A Jr, Guitart JM, et al: Surgical management of intracranial gliomas. **J Neurosurg 19:**365–374, 1962
31. Margetts JC, Kalyan-Raman UP: Giant-celled glioblastoma of brain. A clinico-pathological and radiological study of ten cases

(including immunohistochemistry and ultrastructure). **Cancer 63:** 524–531, 1989
32. Morita M, Rosenblum MK, Bilsky MH, et al: Long-term survivors of glioblastoma multiforme: clinical and molecular characteristics. **J Neurooncol 27:**259–266, 1996
33. Muller H, Brock M, Ernst H: Long-term survival and recurrence-free interval in combined surgical, radio- and chemotherapy of malignant brain gliomas. **Clin Neurol Neurosurg 87:**167–171, 1985
34. Netsky MG, August B, Fowler W: The longevity of patients with glioblastoma multiforme. **J Neurosurg 7:**261–269, 1950
35. New P, Vokes E, Rogers L, et al: Long-term response to crisnatol mesylate in patients with glioma. **Invest New Drugs 15:**343–352, 1997
36. Nomura K: [Current status of treatment of brain tumors in Japan: from the report of brain tumor registry of Japan.] **No To Shinkei 53:**444–452, 2001 (Jpn)
37. Palma E, Celli P, Maleci A, et al: Malignant monstrocellular brain tumours. A study of 42 surgically treated cases. **Acta Neurochir 97:**17–25, 1989
38. Phuphanich S, Ferrall S, Greenberg H: Long-term survival in malignant glioma. Prognostic factors. **J Fla Med Assoc 80:**181–184, 1993
39. Plunkett RJ, Lis A, Barone TA, et al: Hormonal effects on glioblastoma multiforme in the nude rat model. **J Neurosurg 90:** 1072–1077, 1999
40. Pollak L, Gur R, Walach N, et al: Clinical determinants of long-term survival in patients with glioblastoma multiforme. **Tumori 83:**613–617, 1997
41. Puzzilli F, Ruggeri A, Mastronardi L, et al: Long-term survival in cerebral glioblastoma. Case report and critical review of the literature. **Tumori 84:**69–74, 1998
42. Roth JG, Elvidge AR: Glioblastoma multiforme: a clinical survey. **J Neurosurg 17:**736–750, 1960
43. Rutz HP, de Tribolet N, Calmes JM, et al: Long-time survival of a patient with glioblastoma and Turcot's syndrome. Case report. **J Neurosurg 74:**813–815, 1991
44. Sabel M, Reifenberger J, Weber RG, et al: Long-term survival of a patient with giant cell glioblastoma. Case report. **J Neurosurg 94:**605–611, 2001
45. Salcman M, Scholtz H, Kaplan RS, et al: Long-term survival in patients with malignant astrocytoma. **Neurosurgery 34:**213–220, 1994
46. Salford LG, Brun A, Nirfalk S: Ten-year survival among patients with supratentorial astrocytomas grade III and IV. **J Neurosurg 69:**506–509, 1988
47. Salvati M, Cervoni L, Artico M, et al: Long-term survival in patients with supratentorial glioblastoma. **J Neurooncol 36:**61–64, 1998
48. Sant M, van der Sanden G, Capocaccia R: Survival rates for primary malignant brain tumours in Europe. **Eur J Cancer 34:** 2241–2247, 1998
49. Scott JN, Rewcastle NB, Brasher PM, et al: Long-term glioblastoma multiforme survivors: a population-based study. **Can J Neurol Sci 25:**197–201, 1998
50. Scott JN, Rewcastle NB, Brasher PM, et al: Which glioblastoma multiforme patient will become a long-term survivor? A population-based study. **Ann Neurol 46:**183–188, 1999
51. Seki Y, Suico MA, Uto A, et al: The ETS transcription factor MEF is a candidate tumor suppressor gene on the X chromosome. **Cancer Res 62:**6579–6586, 2002
52. Shibamoto Y, Yamashita J, Takahashi M, et al: Supratentorial malignant glioma: an analysis of radiation therapy in 178 cases. **Radiother Oncol 18:**9–17, 1990
53. Surawicz TS, Davis F, Freels S, et al: Brain tumor survival: results from the National Cancer Data Base. **J Neurooncol 40:**151–160, 1998
54. Takeuchi K: [Role of chemotherapy in long-term survival of malignant glioma.] **No Shinkei Geka 3:**815–820, 1975 (Jpn)
55. Takeuchi K, Hoshino K: Statistical analysis of factors affecting survival after glioblastoma multiforme. **Acta Neurochir 37:** 57–73, 1977
56. Taveras JM, Thompson HG Jr, Pool JL: Should we treat glioblastoma multiforme? A study of survival in 425 cases. **AJR 87:** 473–479, 1962
57. Ullen H, Mattsson B, Collins VP: Long-term survival after malignant glioma. A clinical and histopathological study on the accuracy of the diagnosis in a population-based cancer register. **Acta Oncol 29:**875–878, 1990
58. Vertosick FT Jr, Selker RG: Long-term survival after the diagnosis of malignant glioma: a series of 22 patients surviving more than 4 years after diagnosis. **Surg Neurol 38:**359–363, 1992
59. Wester K, Bjerkvig R, Cressey L, et al: Organ culture of a glioblastoma from a patient with an unusually long survival. **Neurosurgery 35:**428–433, 1994
60. Winger MJ, Macdonald DR, Cairncross JG: Supratentorial anaplastic gliomas in adults. The prognostic importance of extent of resection and prior low-grade glioma. **J Neurosurg 71:**487–493, 1989
61. Yoshida T, Kawano N, Oka H, et al: Clinical cure of glioblastoma—two case reports. **Neurol Med Chir 40:**224–229, 2000

Manuscript received October 21, 2003.
Accepted in final form April 2, 2004.
This work was supported by Grant-in-Aid No. 11307025 for Scientific Research from the Ministry of Education, Sports, Science and Culture, Tokyo, Japan, and by Grant No. 1961SC from the Pacific Rim Research Program of the University of California (San Francisco).

Address reprint requests to: Naoki Shinojima, M.D., Department of Neurosurgery, Arao City Hospital, 2600 Arao, Kumamoto 864-0041, Japan. email: shinojima@rio.odn.ne.jp.

CORRELATION BETWEEN PROMOTER HYPERMETHYLATION OF THE O^6-METHYLGUANINE-DEOXYRIBONUCLEIC ACID METHYLTRANSFERASE GENE AND PROGNOSIS IN PATIENTS WITH HIGH-GRADE ASTROCYTIC TUMORS TREATED WITH SURGERY, RADIOTHERAPY, AND 1-(4-AMINO-2-METHYL-5-PYRIMIDINYL)METHYL-3-(2-CHLOROETHYL)-3-NITROSOUREA-BASED CHEMOTHERAPY

Takanori Kamiryo, M.D.
Department of Neurosurgery, Kumamoto University School of Medicine, Kumamoto, Japan

Kenji Tada, M.D.
Department of Neurosurgery, Kumamoto University School of Medicine, Kumamoto, Japan

Shoji Shiraishi, M.D.
Department of Neurosurgery, Kumamoto University School of Medicine, Kumamoto, Japan

Naoki Shinojima, M.D.
Department of Neurosurgery, Kumamoto University School of Medicine, Kumamoto, Japan

Masato Kochi, M.D., Ph.D.
Department of Neurosurgery, Kumamoto University School of Medicine, Kumamoto, Japan

Yukitaka Ushio, M.D., Ph.D.
Department of Neurosurgery, Kumamoto University School of Medicine, Kumamoto, Japan

Reprint requests:
Takanori Kamiryo, M.D., Department of Neurosurgery, Oita Prefectural Hospital, 476 Bunyo, Oita 870-8511, Japan.
Email: tkamiryo-nsu@umin.ac.jp

Received, April 3, 2003.
Accepted, October 7, 2003.

OBJECTIVE: O^6-Methylguanine-deoxyribonucleic acid methyltransferase (MGMT) is a deoxyribonucleic acid repair protein associated with the chemoresistance of chloroethylnitrosoureas. We investigated whether *MGMT* promoter hypermethylation is associated with prognosis in patients with high-grade astrocytic tumors treated uniformly with surgery, radiotherapy, and 1-(4-amino-2-methyl-5-pyrimidinyl)methyl-3-(2-chloroethyl)-3-nitrosourea (ACNU)-based chemotherapy.

METHODS: Using the methylation-specific polymerase chain reaction, we assayed promoter hypermethylation of the *MGMT* gene in tumor deoxyribonucleic acid from 116 adult patients with supratentorial high-grade astrocytic tumors (42 anaplastic astrocytomas [AAs] and 74 glioblastomas multiforme [GBMs]). The Cox proportional-hazards model was used in forward stepwise regression to assess the relative role of prognostic factors (i.e., age at surgery, sex, Karnofsky Performance Scale score, extent of surgical resection, methylation status of the *MGMT* promoter, and association between *MGMT* promoter methylation and survival).

RESULTS: *MGMT* promoter hypermethylation was confirmed in 19 (45.2%) of 42 AA patients and 33 (44.6%) of 74 GBM patients. It was significantly associated with both longer overall and progression-free survival time in AA but not GBM patients.

CONCLUSION: Our results demonstrate that *MGMT* promoter hypermethylation is associated with longer survival time in patients with AA who were treated with surgery, radiotherapy, and ACNU-based chemotherapy, but not in patients with GBM.

KEY WORDS: High-grade astrocytic tumors, *O^6-Methylguanine-deoxyribonucleic acid methyltransferase* gene, Prognosis, Promoter hypermethylation

Neurosurgery 54:349-357, 2004 DOI: 10.1227/01.NEU.0000103422.51382.99 www.neurosurgery-online.com

High-grade astrocytic tumors (anaplastic astrocytoma [AA] and glioblastoma multiforme [GBM]) are the most frequent malignant tumors of the central nervous system. The generally accepted treatment consists of a combination of surgery, radiotherapy, and chemotherapy. Chemotherapy has been shown to modestly increase survival times when used as an adjuvant to surgery and radiation.

Chloroethylnitrosoureas such as 1-(4-amino-2-methyl-5-pyrimidinyl)methyl-3-(2-chloroethyl)-3-nitrosourea (nimustine) (ACNU), 1,3-bis(2-chloroethyl)-1-nitrosourea (carmustine) (BCNU), and 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (lomustine) (CCNU) are commonly used standard chemotherapeutic agents for high-grade astrocytic tumors. They exert their cytotoxic effect(s) on tumor cells by alkylating the O^6 position of guanine, a critical site of alkylation in deoxyribonucleic acid (DNA), resulting in the formation of DNA interstrand cross-links. These covalent bindings result in the inhibition of DNA replication and the induction of tumor cell death (1, 19). In

addition, because chloroethylnitrosoureas can synchronize the cells in the G_2-M phase of the cell cycle, they work as radiosensitizers when combined with radiation therapy. Therefore, the sensitivity of high-grade astrocytic tumors to chloroethylnitrosoureas is of particular interest with respect to treatment choices and outcomes.

One of the best-studied mechanisms of resistance to chloroethylnitrosoureas is the DNA repair protein O^6-methylguanine-DNA methyltransferase (MGMT) (3, 8). MGMT is a ubiquitous DNA repair protein that transfers alkyl adducts from the O^6 position of guanine to an internal cysteine residue before cross-link formation (24), thereby restoring native guanine. Because removal of the adduct results in MGMT inactivation and lesion repair occurs in a stoichiometric fashion, cellular resistance to alkylating agents that produce O^6-guanine adducts is proportional to the number of MGMT molecules present. MGMT protein levels in tumor tissues vary widely by tumor type and even among tumors of similar histology and grade (29). In clinical studies, MGMT expression in tumor tissue was correlated with the therapeutic response to chloroethylating agents, and reduced MGMT expression was associated with prolonged survival (2, 15).

The presence of epigenetic mechanisms of gene silencing in the genesis and progression of human cancers is now firmly established. In cancer cells, genomic methylation patterns are severely disregulated and characterized by overall hypomethylation (especially of repetitive or parasitic elements). This results in genomic instability and paradoxical CpG island hypermethylation associated with concomitant transcriptional gene silencing. Indeed, in human cancers, the latter phenomenon is now thought to be at least as common as classic tumor suppressor gene mutation. In a wide spectrum of human tumors, loss of MGMT activity in tumor cells is not a result of gene deletion or mutation (6, 12, 25) but rather is associated with transcriptional silencing by hypermethylation of the 5' CpG island of *MGMT* (5, 10, 27, 30). Gliomas are tumors with the highest frequency of *MGMT* promoter hypermethylation (10). Esteller et al. (9) demonstrated that in patients with high-grade gliomas manifesting *MGMT* promoter methylation, chemotherapy, including treatment with BCNU, resulted in significantly prolonged survival and progression-free survival. Although BCNU was the drug administered to their patients, another nitrosourea, ACNU, is most widely used in Japan.

We hypothesized that the methylation status of the *MGMT* promoter is also associated with prognosis in high-grade astrocytic tumors treated with ACNU-based chemotherapy and retrospectively studied patients treated uniformly with surgery, radiotherapy, and ACNU-based chemotherapy. Unlike Esteller et al. (9), we found that hypermethylation of the *MGMT* promoter did not confer a survival advantage on GBM patients receiving ACNU-based chemotherapeutic agents. Our study was reviewed and approved by the Human Subjects Review Committee of the University of Kumamoto.

PATIENTS AND METHODS

Patient Population

Our study population consisted of 116 adult patients with primary supratentorial high-grade gliomas involving the hemisphere(s). All were treated with surgery, radiotherapy, and ACNU-based chemotherapy at Kumamoto University Hospital and its affiliated hospitals between November 1989 and December 2001. We excluded patients older than 70 years because in these individuals chemotherapy is contraindicated. The 42 AA patients were 20 men ranging in age from 26 to 68 years (median, 47 yr) and 22 women ranging from 19 to 68 years (median, 43 yr). The 74 GBM patients were 42 men ranging in age from 18 to 69 years (median, 53 yr) and 32 women ranging from 17 to 69 years (median, 52 yr). Written informed consent was obtained from all patients and/or family members before the patients were entered into the study.

Clinical details, including the Karnofsky Performance Scale (KPS) score at the time of diagnosis, were recorded. All patients underwent surgical removal or biopsy sampling of their tumor; the histological diagnosis was confirmed according to World Health Organization criteria (17). The extent of resection was evaluated by early postoperative gadolinium diethylenetriamine penta-acetic acid (Gd-DTPA)-enhanced magnetic resonance imaging (MRI) and classified as gross total resection, subtotal resection, or biopsy (16).

All patients received postoperative adjuvant therapy. A dose of 40 Gy was administered to the high-intensity area plus a 2-cm margin demonstrated on T2-weighted MRI; a boost to a total of 60 Gy was administered to the Gd-DTPA-enhanced area plus a 2-cm margin. In patients without tumor enhancement, a boost of 60 Gy was administered to the high-intensity area plus a 1-cm margin demonstrated on T2-weighted MRI.

Concomitant with radiotherapy, all patients received chemotherapy according to three protocols developed for our prospective randomized trials. Patients whose tumors were diagnosed as AAs received chemotherapy according to Protocol 8702 (used from 1987 to the present), which compared the effectiveness of ACNU (80 mg/m^2 on Day 1, every 6 wk) versus ACNU plus interferon-β (3×10^6 IU 5 times/wk for 6 wk, then 3×10^6 IU every 2 wk). Patients whose tumors were diagnosed as GBM received chemotherapy according to Protocol 8701 (used from 1987 to 1995), which compared the effectiveness of intra-arterial ACNU (80 mg/m^2 on Day 1, every 6 wk) versus intravenous ACNU (18), or according to Protocol 9501 (used from 1995 to the present), which compared the effectiveness of ACNU (70 mg/m^2 on Day 1, every 6 wk), procarbazine (60 mg/m^2 on Days 8–21, every 6 wk), and vincristine (1.4 mg/m^2 on Days 8 and 29, every 6 wk) (PAV) versus PAV plus interferon-β (3×10^6 IU 3 times/wk for 6 wk, then 3×10^6 IU every 2 wk). ACNU is an available chloroethylnitrosourea in Japan, and PAV chemotherapy is an alternative to PCV chemotherapy (CCNU combined with procarbazine and vincristine), in which a low dose of procarbazine is used to potentiate CCNU activity (20, 21).

After initial postoperative therapy, all patients were reexamined. Their KPS score, tumor recurrence or regrowth, onset of clinical deterioration, and death were recorded. Chemotherapy was continued for 1 year or until tumor progression. The survival time was measured as the time from the date of the initial surgery to the date of death. Progression-free survival time was measured from the date of initial surgery to the onset of clinical deterioration or radiologically confirmed tumor recurrence.

Tumor Samples

Tumor tissues were frozen immediately after removal and stored at −80°C until the isolation of genomic DNA. Other tumor tissues were placed in 10% formalin and submitted for histopathological examination. Genomic DNA from the frozen tumors was isolated by proteinase-K digestion and phenol-chloroform extraction.

Analysis of the Methylation Status of the *MGMT* Gene Promoter

The methylation status of the *MGMT* promoter in each tumor was analyzed with the methylation-specific polymerase chain reaction (PCR) assay (14). This method consists of two steps: modification of DNA by sodium bisulfite and PCR amplification. Bisulfite converts unmethylated but not methylated cytosines to uracil bases. The *MGMT* promoter region is then amplified with primers specific for unmethylated and methylated DNA. For bisulfite modification, DNA (1 μg) in a volume of 50 μl was denatured by NaOH (final concentration, 0.2 mol/L) for 20 minutes at 42°C. Then, 30 μl of 10 mmol/L hydroquinone (Sigma Chemical Co., St. Louis, MO) and 520 μl of 3.8 mol/L sodium bisulfite (Sigma Chemical Co.) at pH 5 were added, and the samples were incubated under mineral oil at 55°C for 16 to 20 hours. Modified DNA was purified with Wizard DNA purification resin (Promega, Madison, WI). Modification was completed by 5-minute NaOH treatment (final concentration, 0.3 mol/L) at room temperature, followed by ethanol precipitation. DNA was resuspended in 10 μl of Tris-ethylenediamine tetra-acetic acid buffer. For PCR amplification, the primer sets were as follows: 5'-TTTGTGTTTTGATGTTTGTAGGTTTTTGT-3' (sense) and 5'-AACTCCACACTCTTCCAAAAACAAAACA-3' (antisense) for the unmethylated *MGMT* promoter and 5'-TTTCGACGTTCGTAGGTTTTCGC-3' (sense) and 5'-GCACTCTTCCGAAAACGAAACG-3' (antisense) for the methylated *MGMT* promoter (9). With these primer sets, the amplified region of the *MGMT* promoter spans the area of greatest CpG density immediately 5' to the transcription start site, in an area previously found to be hypermethylated in cells that lacked MGMT activity (10). Normal human lymphocyte DNA was used as a negative control and normal human lymphocyte DNA treated with *SssI* methylase (New England Biolabs, Beverly, MA) as a positive control. PCR amplification was performed in a programmable thermal cycler (MJ Research, Boston, MA) in 25-μl reaction volumes, including 2 μl of bisulfite-modified DNA, 20 mmol/L Tris-HCl, 50 mmol/L KCl, 1.5 mmol/L $MgCl_2$, 200 μmol/L deoxynucleoside triphosphates, 100 ng of each primer, and 2.5 U *Taq* polymerase. After initial denaturation at 95°C for 5 minutes, amplification was carried out for 35 cycles (30 s at 95°C, 30 s at 59°C, and 30 s at 72°C) followed by a final 4-minute extension at 72°C. After PCR, 10 μl of the reaction product was separated by electrophoresis on 3% agarose gels and stained with 0.5 μg/ml ethidium bromide under ultraviolet illumination.

Statistical Analysis

Student's *t* test was used to compare continuous variables. Contingency tables were analyzed by Fisher's exact test. Actuarial survival curves were generated by the Kaplan-Meier method; the log-rank test was used to estimate differences between survival curves. We considered patient age, preoperative KPS score, and extent of tumor resection as clinical factors with a possible effect on the prognosis of patients with high-grade glioma. Because we previously reported sex to be a potential prognostic factor in GBM patients (16), we also included sex in our analyses. The role of these factors in addition to the methylation status of the *MGMT* promoter on overall and progression-free survival was analyzed according to Cox proportional hazards regression. For multivariate analysis, these factors were categorized as follows: age (≤55 yr versus >55 yr), sex (male versus female), preoperative KPS score (≥70% versus <70%), extent of tumor resection (gross total, subtotal, biopsy), and the methylation status of the *MGMT* promoter (unmethylated versus methylated). Factors in multivariate analysis were selected by forward analysis on the basis of maximum partial likelihood estimates. The probability value for entry and removal was 0.2. A probability value of <0.05 was considered statistically significant.

RESULTS

Clinical Characteristics

Preoperative KPS scores ranged from 40 to 100% (median, 90%) in AA patients and from 40 to 100% (median, 80%) in GBM patients. Of the 42 AA patients, 10 underwent gross total resection, 21 subtotal resection, and 11 biopsy. Of the 74 GBM patients, 24 underwent gross total resection, 41 subtotal resection, and 9 biopsy. At the time of this analysis (October 31, 2002), 14 AA patients (33.3%) and 59 GBM patients (79.7%) had died; 18 AA patients (42.9%) and 65 GBM patients (87.8%) had experienced tumor progression. Overall and progression-free survival curves are presented in *Figure 1*.

Methylation of the *MGMT* Promoter

Figure 2 shows representative methylation-specific PCR results. The *MGMT* promoter was methylated in 52 (44.8%) of the 116 high-grade glioma patients, in 19 (45.2%) of 42 AA patients, and in 33 (44.6%) of 74 GBM patients. The methylation incidence by sex was 40.3% (25 of 62) for men and 50.0% (27 of 54) for women ($P = 0.3509$, Fisher's exact test). The mean age of patients with methylated and unmethylated tumors was 48.3 and 48.0 years, respectively ($P = 0.9221$, *t* test). The clinical characteristics of AA and GBM patients as a function of the methylation status of the *MGMT* promoter is shown in *Tables 1* and *2*. The methylation status of the *MGMT* promoter was not significantly corre-

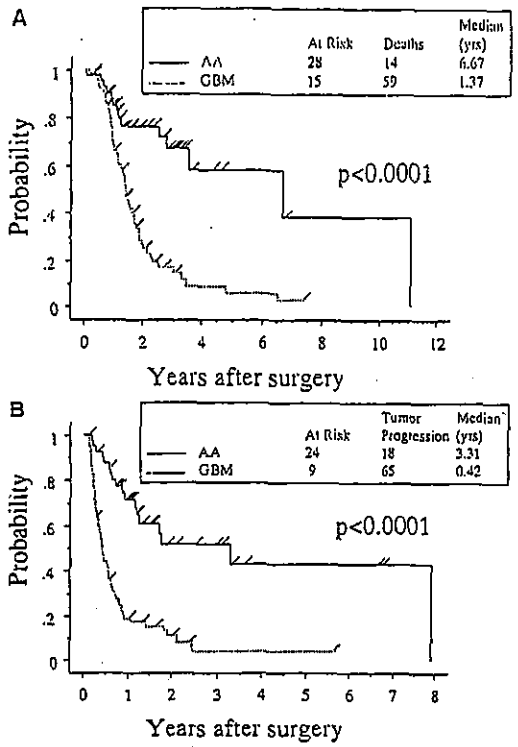


FIGURE 1. *Kaplan-Meier curves of 116 patients with high-grade astrocytic tumors as a function of histological diagnosis. A, overall survival. Number of patients at risk, number of deaths, and median survival times are shown (box). B, progression-free survival. Number of patients at risk, number of patients with disease progression, and median progression-free survival times are shown (box).*

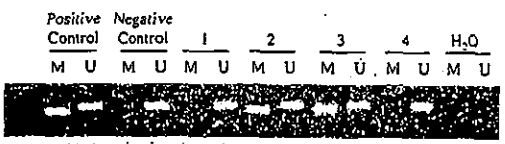


FIGURE 2. *Representative results of methylation-specific PCR assays. The visible PCR product in Lanes U and M indicates the presence of unmethylated and methylated genes, respectively. Samples in Lanes 1 and 4 are from patients without methylation and those in Lanes 2 and 3 from patients with methylation. Normal lymphocyte DNA was the negative and normal lymphocyte DNA treated with SssI methylase, the positive control for methylation. Water was the negative control for PCR.*

lated with any of the selected clinical factors in either the AA or GBM patients. However, in both AA and GBM, the incidence of unmethylated tumors tended to be higher in men.

By univariate analysis, methylation of the *MGMT* promoter was associated with longer overall survival time in all 116 patients ($P = 0.0123$, log-rank test, data not shown). In subset analyses, Kaplan-Meier survival curves showed that AA patients with methylated tumors experienced significantly longer overall survival than did those with unmethylated tumors ($P = 0.0133$, log-rank test) (*Fig. 3A*). In GBM patients, conversely, the methylation status had no significant effect on overall survival (*Fig. 3B*). Similar results were obtained in the analysis of progression-free survival (*Fig. 4, A* and *B*). With respect to overall survival, in AA patients, the only significant factor other than the methylation status of the *MGMT* promoter was the preoperative KPS score; patients with a preoperative score of 70% or more survived longer ($P = 0.0296$, log-rank test). In GBM patients, the only significant factor was age; patients older than 55 years had shorter overall survival ($P = 0.0095$, log-rank test). Although in our study population, sex was not confirmed as a prognostic factor for overall and progression-free survival, we included sex in multivariate analyses to assess the association of survival time with multiple clinical characteristics.

As shown in *Table 3*, the unmethylated *MGMT* promoter status was an independent unfavorable prognostic factor with a strong effect on overall and progression-free survival in AA patients. The risk of death and the risk of disease progression in patients with unmethylated *MGMT* promoter were 13.3 and 4.19, respectively. The unmethylated *MGMT* promoter status had a stronger effect on overall survival than did higher age; it also had a stronger effect on progression-free survival than did the extent of resection. Representative cases of methylated and unmethylated *MGMT* promoter are shown in *Figures 5* and 6, respectively.

Conversely, in GBM patients, the methylation status of the *MGMT* promoter was not a predictive variable with respect to either overall or progression-free survival (*Table 4*). Multivariate analysis showed that the only significant prognostic factors for overall survival were higher age and male sex.

DISCUSSION

Our study population consisted of adult patients with frozen tumor samples sufficiently large for the analysis of methylation of the *MGMT* promoter who had been enrolled in one of our three prospective randomized trials. Hypermethylation of the *MGMT* gene promoter was detected by methylation-specific PCR assay, the most widely used technique for studying hypermethylation of CpG islands (14). Hypermethylation of the promoter of the *MGMT* gene is the epigenetic mechanism that represses transcriptional activity in cancer cells and is not found in normal cells (10). This tumor specificity is important: clinical tissue samples of gliomas contain nontumor cells or normal cells because gliomas invade the surrounding brain parenchyma diffusely. Because methylation-specific PCR sensitively detects the methylation status of small clinical specimens such as needle biopsy samples, we chose this method. Although microdissection-assisted DNA extraction may be required because the PCR assay we used may not detect hypermethylation in a small fraction of tumor cells, microscopic examination confirmed the presence of viable tumor cells in all our samples. The frequency of *MGMT* promoter methylation in our study (AA, 45.2%; GBM, 44.6%) approximated that reported by Esteller et al. (9) (AA, 39%; GBM, 41%).

It remains controversial whether the MGMT protein level in primary tumors is predictive of a response to the

TABLE 1. Clinical characteristics of 42 anaplastic astrocytoma patients as a function of their *O⁶-methylguanine-deoxyribonucleic acid methyltransferase* promoter methylation status[a]

Factor	Unmethylated (n = 23)		Methylated (n = 19)		*P* value
	No.	%	No.	%	
Age (yr)					0.7483
≤55	16	70	12	63	
>55	7	30	7	37	
Sex					0.2321
Male	13	57	7	37	
Female	10	43	12	63	
Preoperative KPS score (%)					0.6729
≥70	19	83	17	89	
<70	4	17	2	11	
Extent of resection					0.3049
Gross total resection	7	30	3	16	
Subtotal resection and biopsy	16	70	16	84	

[a] KPS, Karnofsky Performance Scale.

TABLE 2. Clinical characteristics of 74 glioblastoma patients as a function of their *O⁶-methylguanine-deoxyribonucleic acid methyltransferase* promoter methylation status[a]

Factor	Unmethylated (n = 41)		Methylated (n = 33)		*P* value
	No.	%	No.	%	
Age (yr)					0.6194
≤55	29	71	21	64	
>55	12	29	12	36	
Sex					0.8150
Male	24	59	18	55	
Female	17	41	15	45	
Preoperative KPS score (%)					0.5802
≥70	31	76	27	82	
<70	10	24	6	18	
Extent of resection					0.4595
Gross total resection	15	37	9	27	
Subtotal resection and biopsy	26	63	24	73	

[a] KPS, Karnofsky Performance Scale.

chloroethylnitrosourea-based chemotherapy commonly used in the treatment of high-grade gliomas. Silber et al. (29) analyzed 43 high-grade gliomas (19 AAs and 24 GBMs) by biochemical assay of tumor tissue extracts and compared survival between patients with and without MGMT activity. In their study, MGMT activity was higher in tumors that recurred after chemotherapy including BCNU or after PCV chemotherapy than in tumors that recurred in patients who had not received chemotherapy. Their findings suggest that tumor cells without MGMT activity were killed selectively in situ by BCNU- or CCNU-based chemotherapy. However, the level of MGMT activity in tumors after chemotherapy did not affect progression-free survival. Belanich et al. (2), in a retrospective study of primary brain tumors, examined the correlation between MGMT protein levels detected by quantitative immunofluorescence assay and survival in 99 GBM patients treated with surgery, radiotherapy, and BCNU-based chemotherapy. Unlike Silber et al. (29), they found that a low level of MGMT

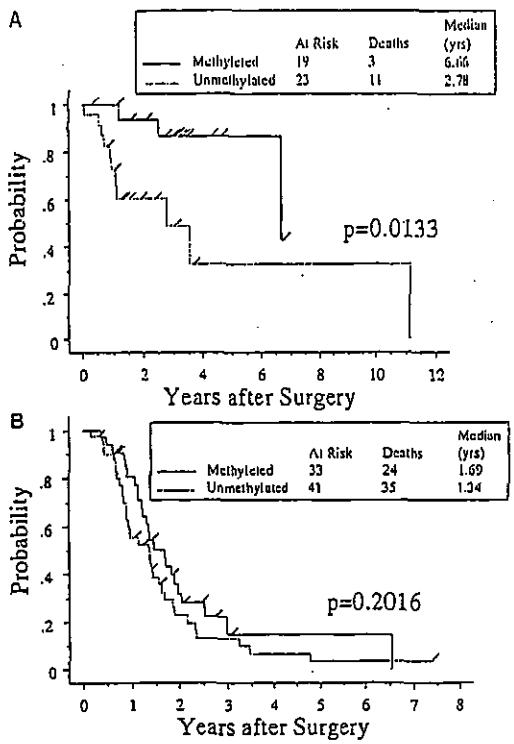


FIGURE 3. *Kaplan-Meier curves for overall survival of (A) 42 AA patients and (B) 74 GBM patients as a function of the MGMT promoter methylation status. Number of patients at risk, number of deaths, and median survival times are shown (boxes).*

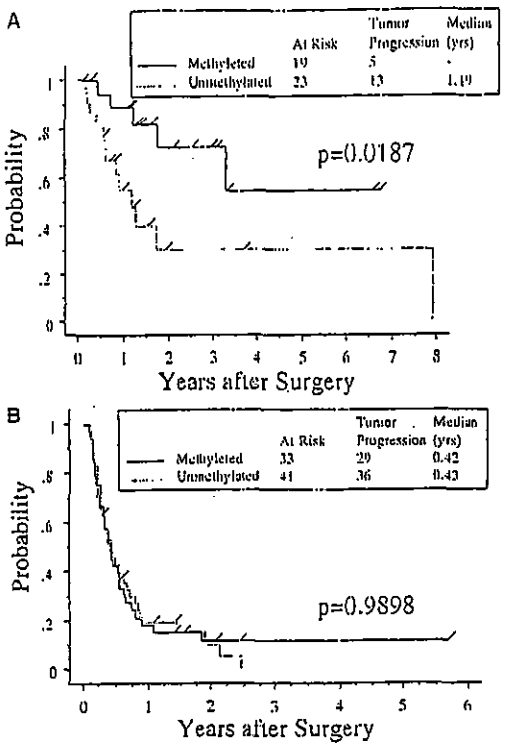


FIGURE 4. *Kaplan-Meier curves for progression-free survival of (A) 42 AA patients and (B) 74 GBM patients as a function of the MGMT promoter methylation status. Number of patients at risk, number of patients with disease progression, and median progression-free survival times are shown (boxes).*

was significantly associated with longer overall and progression-free survival. Similarly, Jaeckle et al. (15) observed a correlation between MGMT levels and survival in 64 patients with high-grade gliomas (24 AAs and 40 GBMs) treated with surgery, radiotherapy, and BCNU; patients whose tumors manifested low MGMT levels had significantly longer overall and progression-free survival. Their subset analysis by astrocytoma grade showed that in 24 AA patients, the median survival of patients with high MGMT levels was 14 months, compared with 62 months for those with low MGMT levels. The median survival in their group of 40 GBM patients was 7 months for patients with tumors manifesting high MGMT levels compared with 12 months for those with low MGMT levels. However, they did not subject their results to statistical analysis.

TABLE 3. Multivariate analysis in 42 anaplastic astrocytoma patients[a]

Variable	Overall survival			Progression-free survival		
	P value[b]	Hazard ratio	95% confidence interval	*P* value[b]	Hazard ratio	95% confidence interval
Age >55 yr	0.0053	6.77	1.76–26.0	–	–	–
Male	–	–	–	0.1279	0.45	0.163–1.255
Preoperative KPS score ≥70%	0.1313	0.33	0.079–1.391	–	–	–
Gross total resection	–	–	–	0.0366	0.43	0.193–0.948
MGMT promoter, unmethylated	0.0020	13.3	2.58–69.0	0.0089	4.19	1.43–12.3

[a] –, not retained in the final model; KPS, Karnofsky Performance Scale; *MGMT*, *O*^{6}*-methylguanine-deoxyribonucleic acid methyltransferase* gene.
[b] *P* values of <0.05 were considered significant.

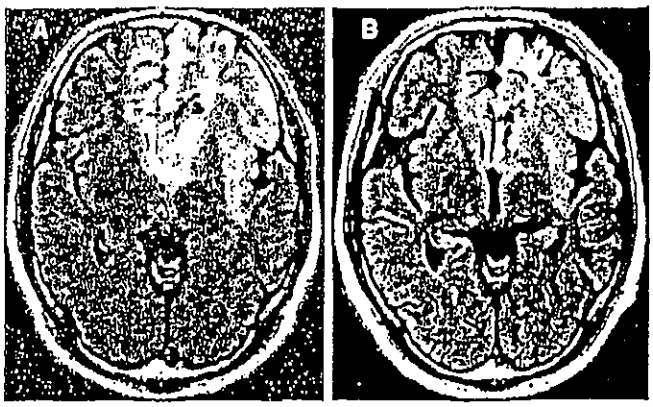


FIGURE 5. *MRI scans in a representative methylated* MGMT *promoter case. This 43-year-old man, who had a generalized tonic seizure, underwent stereotactic biopsy of a left frontal AA on February 4, 1999 (A). After radiation (60 Gy) and five courses of chemotherapy (ACNU/interferon-β), the tumor, which was hyperintense on T2-weighted MRI scans but not on Gd-DTPA-enhanced MRI scans, was decreased in size (B).*

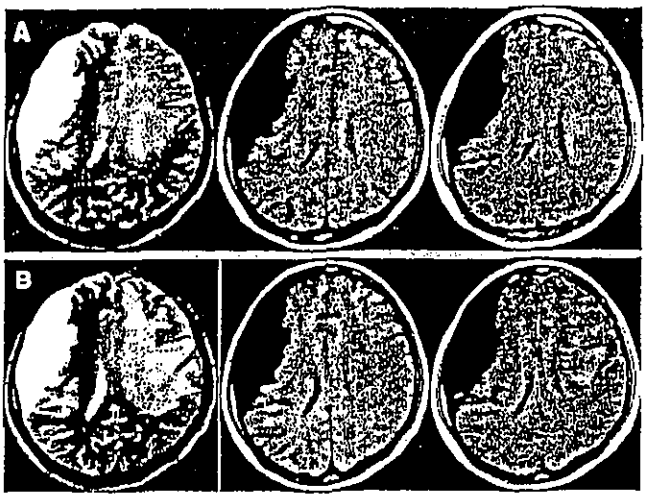


FIGURE 6. *MRI scans in a representative unmethylated* MGMT *promoter case. This 42-year-old woman with right numbness and weakness underwent stereotactic biopsy of an AA of the left corona radiata on June 17, 1999 (A). After radiation (60 Gy) and three courses of chemotherapy (ACNU/interferon-β), she experienced a partial seizure. MRI revealed tumor progression (B). The right extra-axial lesion is an arachnoid cyst.*

In their analysis of 47 high-grade gliomas (18 AAs and 29 GBMs), Esteller et al. (9) found that *MGMT* promoter methylation was associated with responsiveness to chemotherapy, including BCNU. They suggested that the methylation status might be useful for predicting the chemosensitivity of gliomas. However, because their chemotherapeutic regimen included cisplatin as well as BCNU, the effect of BCNU alone could not be determined. All patients in the present study were grouped according to histological diagnosis (AA or GBM), and all were treated with ACNU-based chemotherapy. We analyzed survival for the two groups separately and found a previously unreported association between the *MGMT* promoter methylation status and prognosis in patients with high-grade astrocytic tumor.

Unlike those of Belanich et al. (2), our findings suggest that *MGMT* promoter methylation is not a prognostic factor in GBM patients. However, because the two studies differed in several respects, direct comparison of the results is not possible. Our patients received ACNU-based chemotherapy, whereas theirs were treated with BCNU-based chemotherapy. In addition, the target of detection was different; we studied hypermethylation of the gene promoter, whereas they focused on the MGMT protein level in tumors. We speculate that the resistance of GBM to our ACNU-based chemotherapy may be attributable to yet unknown factors in addition to the MGMT level of the tumors.

According to multivariate analysis, *MGMT* promoter methylation was a statistically significant prognostic factor in AA patients. It was a significant covariate that surpassed other clinically important prognostic factors such as age, the preoperative KPS score, and the extent of tumor resection. We posit that this may reflect a wide spectrum of chemosensitivity and diverse clinical outcomes in AAs, considered intermediate-grade astrocytic tumors that vary with respect to overall and

TABLE 4. Multivariate analysis in 74 glioblastoma patients[a]

Variable	Overall survival			Progression-free survival		
	P value[b]	Hazard ratio	95% confidence interval	*P* value[b]	Hazard ratio	95% confidence interval
Age >55 yr	0.0036	2.33	1.32–4.12	0.1721	1.47	0.846–2.546
Male	0.0391	1.78	1.03–3.07	–	–	–
Preoperative KPS score ≥70%	–	–	–	0.0521	0.56	0.311–1.005
Gross total resection	–	–	–	–	–	–
MGMT promoter, unmethylated	–	–	–	–	–	–

[a] –, not retained in the final model; KPS, Karnofsky Performance Scale; *MGMT*, *O*^{6}*-methylguanine-deoxyribonucleic acid methyltransferase* gene.
[b] *P* values of <0.05 were considered significant.

progression-free survival from several months (similar to GBMs) to several years (similar to low-grade astrocytomas).

In our GBM patients, male sex was an independent indicator of a poor prognosis. Some studies have reported that among glioma patients, women had a better prognosis (22, 23). Female hormones seemed to be associated with longer survival in a rat model of GBM (26) and with a lower incidence of glioma in women in a population-based case-control study (28). According to a retrospective study of AA patients treated with radiotherapy and nitrosourea-based chemotherapy, women manifested longer survival than men (7). We suggest that sex should be considered in assessing the survival of patients with high-grade gliomas.

Chloroethylnitrosoureas are the most widely used chemotherapeutic agents for high-grade gliomas because of their high permeation through the blood-brain barrier. However, these agents have failed to improve survival dramatically, and conventional treatment with surgery, radiotherapy, and chloroethylnitrosourea-based chemotherapy fails to cure all patients with GBM and most patients with AA (4, 11, 13). In the absence of more effective treatment strategies, the type of information yielded by our study may help the clinician in identify patients who should receive aggressive chemotherapy and those who should be treated by alternative therapies.

REFERENCES

1. Aida T, Bodell WJ: Effect of caffeine on cytotoxicity and sister chromatid exchange induction in sensitive and resistant rat brain tumor cells treated with 1,3-bis(2-chloroethyl)-1-nitrosourea. Cancer Res 47:5052–5058, 1987.
2. Belanich M, Pastor M, Randall T, Guerra D, Kibitel J, Alas L, Li B, Citron M, Wasserman P, White A, Eyre H, Jaeckle K, Schulman S, Rector D, Prados M, Coons S, Shapiro W, Yarosh D: Retrospective study of the correlation between the DNA repair protein alkyltransferase and survival of brain tumor patients treated with carmustine. Cancer Res 56:783–788, 1996.
3. Bobola MS, Berger MS, Silber JR: Contribution of O^6-methylguanine-DNA methyltransferase to resistance to 1,3-(2-chloroethyl)-1-nitrosourea in human brain tumor-derived cell lines. Mol Carcinog 13:81–88, 1995.
4. Chang CH, Horton J, Schoenfeld D, Salazer O, Perez-Tamayo R, Kramer S, Weinstein A, Nelson JS, Tsukada Y: Comparison of postoperative radiotherapy and combined postoperative radiotherapy and chemotherapy in the multidisciplinary management of malignant gliomas: A joint Radiation Therapy Oncology Group and Eastern Cooperative Oncology Group Study. Cancer 52:997–1007, 1983.
5. Costello JF, Berger MS, Huang HS, Cavenee WK: Silencing of p16/CDKN2 expression in human gliomas by methylation and chromatin condensation. Cancer Res 56:2405–2410, 1996.
6. Day RS III, Ziolkowski CH, Scudiero DA, Meyer SA, Lubiniecki AS, Girardi AJ, Galloway SM, Bynum GD: Defective repair of alkylated DNA by human tumour and SV40-transformed human cell strains. Nature 288:724–727, 1980.
7. Diete S, Treuheit T, Dietzmann K, Schmidt U, Wallesch CW: Sex differences in length of survival with malignant astrocytoma, but not with glioblastoma. J Neurooncol 53:47–49, 2001.
8. Erickson LC, Laurent G, Sharkey NA, Kohn KW: DNA cross-linking and monoadduct repair in nitrosourea-treated human tumour cells. Nature 288: 727–729, 1980.
9. Esteller M, Garcia-Foncillas J, Andion E, Goodman SN, Hidalgo OF, Vanaclocha V, Baylin SB, Herman JG: Inactivation of the DNA-repair gene MGMT and the clinical response of gliomas to alkylating agents. N Engl J Med 343:1350–1354, 2000.
10. Esteller M, Hamilton SR, Burger PC, Baylin SB, Herman JG: Inactivation of the DNA repair gene O^6-methylguanine-DNA methyltransferase by promoter hypermethylation is a common event in primary human neoplasia. Cancer Res 59:793–797, 1999.
11. Fine HA: The basis for current treatment recommendations for malignant gliomas. J Neurooncol 20:111–120, 1994.
12. Fornace AJ Jr, Papathanasiou MA, Hollander MC, Yarosh DB: Expression of the O^6-methylguanine-DNA methyltransferase gene MGMT in MER^+ and MER^- human tumor cells. Cancer Res 50:7908–7911, 1990.
13. Green SB, Byar DP, Walker MD, Pistenman DA, Alexander E Jr, Batzdorf U, Brooks WH, Hunt WE, Mealey J Jr, Odom GL, Paoletti P, Ransohoff J II, Robertson JT, Selker RG, Shapiro WR, Smith KR Jr, Wilson CB, Strike TA: Comparisons of carmustine, procarbazine, and high-dose methylprednisolone as additions to surgery and radiotherapy for the treatment of malignant glioma. Cancer Treat Rep 67:121–132, 1983.
14. Herman JG, Graff JR, Myohanen S, Nelkin BD, Baylin SB: Methylation-specific PCR: A novel PCR assay for methylation status of CpG islands. Proc Natl Acad Sci U S A 93:9821–9826, 1996.
15. Jaeckle KA, Eyre HJ, Townsend JJ, Schulman S, Knudson HM, Belanich M, Yarosh DB, Bearman SI, Giroux DJ, Schold SC: Correlation of tumor O^6-methylguanine-DNA methyltransferase levels with survival of malignant astrocytoma patients treated with bis-chloroethylnitrosourea: A Southwest Oncology Group Study. J Clin Oncol 16:3310–3315, 1998.
16. Kamiryo T, Tada K, Shiraishi S, Shinojima N, Nakamura H, Kochi M, Kuratsu J, Saya H, Ushio Y: Analysis of homozygous deletion of the p16 gene and correlation with survival in patients with glioblastoma multiforme. J Neurosurg 96:815–822, 2002.
17. Kleihues P, Cavenee WK: *Pathology and Genetics of Tumours of the Nervous System*. Lyon, IARC Press, 2000.
18. Kochii M, Kitamura I, Goto T, Nishi T, Takeshima H, Saito Y, Yamamoto K, Kimura T, Kino T, Tada K, Shiraishi S, Uemura S, Iwasaki T, Kuratsu J, Ushio Y: Randomized comparison of intra-arterial versus intravenous infusion of ACNU for newly diagnosed patients with glioblastoma. J Neurooncol 49:63–70, 2000.
19. Kohn KW: Interstrand cross-linking of DNA by 1,3-bis(2-chloroethyl)-1-nitrosourea and other 1-(2-haloethyl)-1-nitrosoureas. Cancer Res 37:1450–1454, 1977.
20. Levin VA, Wilson CB: Correlations between experimental chemotherapy in the murine glioma and effectiveness of clinical therapy regimens. Cancer Chemother Pharmacol 1:41–48, 1978.
21. Levin VA, Edwards MS, Wright DC, Seager ML, Schimberg TP, Townsend JJ, Wilson CB: Modified procarbazine, CCNU, and vincristine (PCV 3) combination chemotherapy in the treatment of malignant brain tumors. Cancer Treat Rep 64:237–244, 1980.
22. McKeever PE, Ross DA, Strawderman MS, Brunberg JA, Greenberg HS, Junck L: A comparison of the predictive power for survival in gliomas provided by MIB-1, bromodeoxyuridine and proliferating cell nuclear antigen with histopathologic and clinical parameters. J Neuropathol Exp Neurol 56:798–805, 1997.
23. North CA, North RB, Epstein JA, Plantadosi S, Wharam MD: Low-grade cerebral astrocytomas: Survival and quality of life after radiation therapy. Cancer 66:6–14, 1990.
24. Pegg AE, Dolan ME, Moschel RC: Structure, function, and inhibition of O^6-alkylguanine-DNA alkyltransferase. Prog Nucleic Acid Res Mol Biol 51:167–223, 1995.
25. Pieper RO, Futscher BW, Dong Q, Ellis TM, Erickson LC: Comparison of O^6-methylguanine DNA methyltransferase (MGMT) mRNA levels in Mer^+ and Mer^- human tumor cell lines containing the MGMT gene by the polymerase chain reaction technique. Cancer Commun 2:13–20, 1990.
26. Plunkett RJ, Lis A, Barone TA, Fronckowiak MD, Greenberg SJ: Hormonal effects on glioblastoma multiforme in the nude rat model. J Neurosurg 90:1072–1077, 1999.
27. Qian XC, Brent TP: Methylation hot spots in the 5' flanking region denote silencing of the O^6-methylguanine-DNA methyltransferase gene. Cancer Res 57:3672–3677, 1997.
28. Schlehofer B, Blettner M, Wahrendorf J: Association between brain tumors and menopausal status. J Natl Cancer Inst 84:1346–1349, 1992.

29. Silber JR, Blank A, Bobola MS, Ghatan S, Kolstoe DD, Berger MS: O^6-Methylguanine-DNA methyltransferase-deficient phenotype in human gliomas: Frequency and time to tumor progression after alkylating agent-based chemotherapy. Clin Cancer Res 5:807–814, 1999.
30. Watts GS, Pieper RO, Costello JF, Peng YM, Dalton WS, Futscher BW: Methylation of discrete regions of the O^6-methylguanine DNA methyltransferase (MGMT) CpG island is associated with heterochromatinization of the MGMT transcription start site and silencing of the gene. Mol Cell Biol 17:5612–5619, 1997.

Acknowledgments

We thank Dr. Akira Kitagawa, Department of Pharmaceutics, Kumamoto University Medical School Hospital, for help with the statistical analyses. This work was supported by Grants-in-aid for Scientific Research from the Ministry of Education, Sports, Science, and Culture of Japan and by Grants-in-aid from the Pacific Rim Research Program (USA).

COMMENTS

There are a number of differences between anaplastic gliomas and glioblastoma, including prognosis and response to therapy. These establish clearly different prognostic classes of high-grade gliomas. The authors demonstrate another difference in their report showing that hypermethylation of the *MGMT* gene carries a better prognosis for anaplastic astrocytomas that does not occur in glioblastomas. This is a nice report, and the data raise some interesting questions for further study.

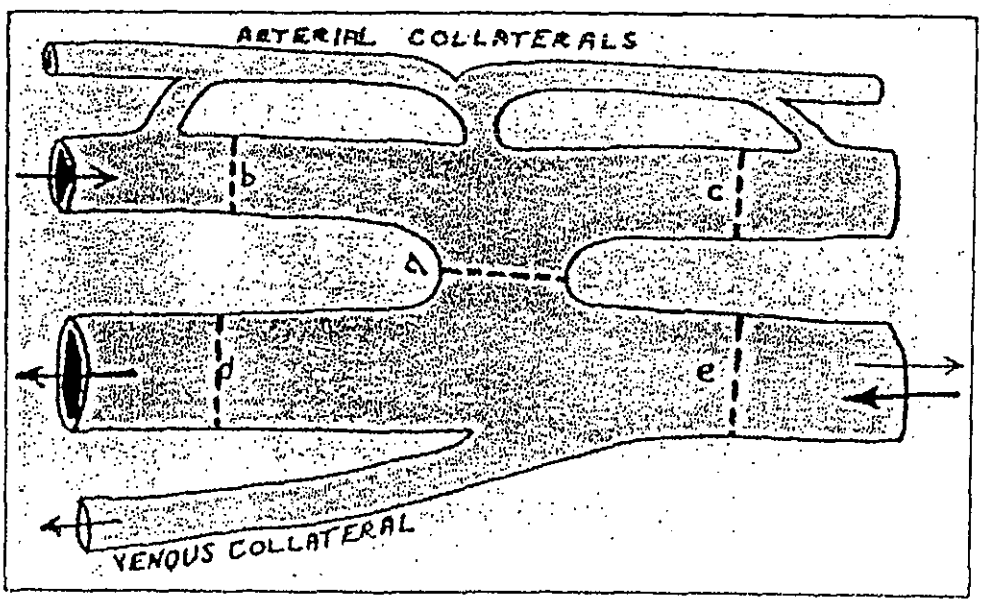
Joseph M. Piepmeier
New Haven, Connecticut

Kamiryo et al. show that aberrant methylation of selected CpG sites in the *MGMT* gene correlates with a better prognosis in patients with anaplastic astrocytoma, but it is not a significant prognostic factor in patients with glioblastoma multiforme. Previously, Esteller et al. (1) provided the first demonstration that *MGMT* methylation is a useful predictor of overall and progression-free survival in malignant gliomas (both anaplastic astrocytoma and glioblastoma multiforme groups combined). In addition, other studies that tested for correlations between MGMT protein activity in malignant gliomas and overall survival have shown conflicting results. The study by Kamiryo et al. is important in addressing the controversy and in providing an independent assessment of the conclusions from the study by Esteller et al.

Any comparison of these studies is necessarily limited by the differences in treatments and patient populations. The 116 patients in the study by Kamiryo et al. received surgery, local radiation, and nimustine-based chemotherapy. The 47 patients in the study by Esteller et al. were treated with surgery, whole-brain irradiation, and both cisplatin- and carmustine-based chemotherapy. In contrast, the analysis of *MGMT* methylation was performed in an apparently identical fashion between these two studies, and the overall incidence of methylation was quite similar. In both studies, the analysis is well controlled, and samples are defined as methylated by the presence of a polymerase chain reaction product from the M primer set. Direct comparison of these two methylation studies with those that measure *MGMT* activity is more problematic. The presence of some level of aberrant methylation is not necessarily a direct reflection of the MGMT protein level, because there are a number of steps in between, and there are also considerations of the differences in quantitative aspects of the measurement of *MGMT* methylation and MGMT protein activity. Kamiryo et al. present a balanced view of these studies and avoid undue comparisons.

Joe Costello
Mitchel S. Berger
San Francisco, California

1. Esteller M, Garcia-Foncillas J, Andion E, Goodman SN, Hidalgo OF, Vanaclocha V, Baylin SB, Herman JG: Inactivation of the DNA-repair gene MGMT and the clinical response of gliomas to alkylating agents. N Engl J Med 343:1350–1354, 2000.



Diagrammatic representation of ligations used to control an arteriovenous aneurysm (from, Hamby WB, Gardner WJ: Treatment of pulsating exophthalmos with report of two cases. Arch Surg 27:676–685, 1933). Also see pages 437 and 479.

J Neurosurg 100:1101–1106, 2004

Gradual recovery from dyslexia and related serial magnetoencephalographic changes in the lexicosemantic centers after resection of a mesial temporal astrocytoma

Case report

KYOUSUKE KAMADA, M.D., YUTAKA SAWAMURA, M.D., FUMIYA TAKEUCHI, PH.D., KIYOHIRO HOUKIN, M.D., HIDEAKI KAWAGUCHI, M.D., YOSHINOBU IWASAKI, M.D., AND SHINYA KURIKI, PH.D.

Department of Neurosurgery, Research Institute for Electronic Science, Department of Laboratory Medicine, Hokkaido University; and Department of Neurosurgery, Sapporo Medical College, Sapporo, Japan

✓ Letter-perception centers are not held in as high regard as motor- and language-related cortices during planning of neurosurgical procedures, and there have been no reports suggesting cortical reorganization of reading ability. The authors describe a patient with a left mesial temporal glioma in whom two letter-perception centers (the anterior portion of the left superior temporal gyrus and the left fusiform gyrus) were successfully localized before surgery by performing magnetoencephalography (MEG) during reading tasks. Control MEG examinations of 15 healthy volunteers were also performed to assist in a careful interpretation of patient results. Although a radical resection of the mesial temporal glioma, which involved the left fusiform gyrus, caused severe dyslexia, the patient's impaired reading skills improved gradually during a 1-year postoperative period. In the meantime, the spared left superior temporal gyrus displayed an overshot recovery of MEG responses. During the postoperative period there was no obvious recovery in MEG signals and no compensatory activity in the contralateral fusiform gyrus. This case demonstrates that lexicosemantic centers involved in the reading process can be noninvasively localized using MEG and that the results obtained are highly reliable for surgical planning. The results of the repeated MEG reflected sequentially the patient's recovery from dyslexia. This is the first report in which MEG studies have been shown to predict preoperatively the risk of dyslexia and demonstrate its serial physiological recovery.

KEY WORDS • dyslexia • glioma • mesial temporal lobe • functional recovery • magnetoencephalography

It has been generally accepted that resections performed in the language-dominant hemisphere must spare the classic frontal motor and temporal receptive language areas to avoid persistent aphasia. Most neurosurgeons have strictly followed this basic guideline, even in glioma surgery.

On the other hand, the cortical areas related to letter perception have not been well acknowledged. The semantic center of letter reading has not been seriously considered during surgical planning. Reading impairments such as dyslexia can be masked by coexistent major complications such as aphasia or dementia. Nevertheless, dyslexia essentially affects patients' intellectual life and work and is a subject that should be duly considered as a major complication.

Previous studies of lesions have demonstrated that the superior temporal and angular gyri in the dominant hemisphere contribute mainly to letter-reading processes.[3,6] Nobre, et al.,[11] found the site of lexicosemantic activity related to the reading process in the inferior temporal region, including the fusiform gyrus, by using subdural electrodes. Studies in healthy volunteers in which noninvasive functional imaging techniques such as PET and fMR imaging were performed have demonstrated that the inferior temporal area is activated by several reading tasks.[1,13,15] The functional dominance of the fusiform gyrus, however, remains unclear because most studies have demonstrated bilateral responses in reading processes.

Magnetoencephalography directly detects neuronal activity and provides a better time resolution than other noninvasive methods of mapping. In this report, an MEG investigation in which letter-reading tasks were performed preoperatively localized the lexicosemantic centers in a patient with a left mesial temporal glioma. After complete resection of the tumor, which involved the inferomesial temporal region in which the semantic-MEG dipoles were concentrated, the patient suffered from severe dyslexia. He gradually recovered within 1 year. We followed the patient's recovery by performing serial MEG, which reflected the severity of the dyslexia and the clinical usefulness of lexicosemantic MEG.

Abbreviations used in this paper: fMR = functional magnetic resonance; IQ = intelligence quotient; LORETA = low-resolution electromagnetic tomography; MEG = magnetoencephalography; PET = positron emission tomography; RMS = root mean square; SLTA = Standard Language Test for Aphasia; WAIS-R = Wechsler Adult Intelligence Test-Revised.

TABLE 1

*Results of the SLTA and lexicosemantic MEG investigations**

Timing	SLTA Score (%)				MEG	
	Reading	Writing	Speech	Verbal Comprehension	Mean Reaction Time (msec)†	Task Performance (%)
preop	100	100	100	100	800 ± 213	92.3
7 or 10 days‡	42	75	78	80	NT	NT
3 mos	80	88	90	90	1320 ± 379§	63.8
8 mos	90	92	97	98	1212 ± 305§	83.0

* NT = not tested.
† Mean ± standard deviation.
‡ Neuropsychological tests and the MEG study were performed 7 and 10 days postoperatively, respectively.
§ $p < 0.5$, Student t-test.

Case Report

History. This 34-year-old, right-handed man experienced transient amnesia for a few minutes in April 2001. Before the incident he had done well in his employment as an office worker. Neurological examination revealed no abnormality on the day after the episode, but T_1-weighted MR images revealed a large hypointense mass in the left mesial temporal region. The lesion appeared homogeneously hyperintense on T_2-weighted MR images and was not enhanced following a Gd–diethylenetriamine pentaacetic acid injection. The mass involved the hippocampus, uncus, amygdala, and parahippocampal and fusiform gyri, but not the superior or middle temporal gyri. These findings suggested that the mass was a low-grade astrocytoma originating from the mesial temporal lobe. No neurological deficit had appeared before treatment and thus our major concern was whether the brain area to be involved in surgery would still function postoperatively.

Examination. Preoperative neuropsychological examinations, including the SLTA (Japanese edition), WAIS-R, Miyake auditory–verbal memory test, and Benton Visual Retention Test detected no language deficits or memory disturbance. The SLTA is the standardized test battery most commonly used to evaluate Japanese patients with aphasia. The aphasia severity ratings (range 0, most severe–10, normal) are based on the 19 subscores of the SLTA, and these were used as a primary language measurement for this patient. The following six subscores of the SLTA were sequentially analyzed: reading aloud words; reading comprehension (in which the patient points out images of objects indicated by written words); dictation of letters; naming; auditory comprehension (ability to obey verbal commands); and sentence repetition. The patient could complete the tasks of the SLTA without difficulty and obtained full points for all the subscores. The verbal and performance IQs, determined using the WAIS-R, were 112 and 118, respectively. The patient's hand preference was predominantly right sided (+105 on the Edinburgh Handedness Inventory),[12] and an intracarotid sodium amobarbital test (Wada test) revealed a left-hemisphere dominance for language functions and a right-hemisphere dominance for memory. Lexicosemantic MEG, performed using a letter-reading task, localized two letter-perception centers (the anterior portion of the left superior temporal gyrus and the left fusiform gyrus), as described in detail later in this paper. Because this large low-grade glioma was thought to be life threatening, but curable by a complete resection, we proposed radical removal of the tumor involving the inferior temporal region, informing the patient of the risks of possible postsurgical neurological deficits. The patient accepted the treatment plan and gave his informed consent to participate in pre- and postoperative lexicosemantic examinations including MEG and neuropsychological tests.

Operation. The middle and inferior temporal gyri were exposed by a frontotemporal craniotomy. The brain tumor was found after a corticotomy, which encompassed a 4-cm anterior portion of the inferior temporal gyrus. Intraoperative observation disclosed tumor invasion into the inferior temporal gyrus, fusiform gyrus, amygdala, uncus, and hippocampus. The involved brain tissue was completely resected. The histopathological diagnosis was World Health Organization Grade II diffuse astrocytoma.

Postoperative Course. The patient awoke with severe dyslexia and a slight receptive aphasia. Auditory comprehension and repetition and naming capabilities were almost intact. Neurological and neuropsychological examinations were serially performed throughout an 8-month postoperative period. On the 7th postoperative day, the man still displayed severe reading and writing impairments (scores of 3 in reading aloud, 4 in reading comprehension, and 7 in letter dictation; Table 1) with a right upper homonymous quadrantanopia. Speech function and auditory comprehension were, however, relatively preserved. The man's verbal IQ (WAIS-R) was 88, which was lower than preoperatively despite the fact that he retained a normal performance IQ (116).

Three months after surgery, the impairments had improved, but he still had difficulty in reading and exhibited phonemic paralexia (scores of 6 in reading aloud and 8 in reading comprehension). It is noteworthy that he could point out objects correctly with a finger, even though he could not read aloud the names of written objects (understanding without phonology) (Table 1). Eight months after surgery, the patient's reading impairment had remarkably improved (scores of 8.5 in reading aloud and 9 in reading comprehension) and he became able to read newspapers with some effort. His verbal IQ (103) was much improved, but did not reach his preoperative level.

The Miyake auditory–verbal memory test and the Benton Visual Retention Test did not show any deterioration in the patient's short-term memory and, clinically, he displayed little memory disturbance following the operation. He returned to his office work, but still acknowledged lingering reading difficulties.

Summary of Tests and Findings

Lexicosemantic MEG Studies

The MEG signals were recorded using a 204-channel biomagnetometer (VectorView; Neuromag, Helsinki, Finland) in a magnetically shielded room. Serial MEG studies were performed before the operation and 10 days, 3 months, and 8 months after surgery. Despite the fact that the patient's reading comprehension skills generally improved throughout the postoperative period, the postoperative MEG findings were compared with the preoperative MEG findings. We acquired two data sets for each task to confirm stable and consistent MEG responses. In particular, we performed the preoperative MEG investigations on two different days (7 and 3 days before surgery) and also performed control examinations in 15 strongly right-handed volunteers who had experienced no adverse cerebral events or neurological deficits.

One hundred fifty words were visually presented with a 300-msec exposure time and interstimulus intervals ranging between 2800 and 3200 msec during the MEG recordings. Each word was a noun that consisted of three kana letters (Japanese phonetic symbols that were presented, centered at a 4° visual angle). The patient and volunteers were asked to categorize the presented word as abstract or concrete by pushing buttons with the index or middle finger, respectively (kana reading). To identify the lexicosemantic response specific to the kana-reading task, we presented 150 pairs of Arabic letters and asked the patient and volunteers to decide whether each pair had the same letters or different ones (figure discrimination). All volunteers and the patient received instructions and were allowed brief practice sessions before the measurement.

Each epoch consisted of a 500-msec prestimulus baseline and a 1500-msec analysis period following stimulus delivery. One hundred fifty epochs of magnetic signals were averaged and digitally filtered between 0.5 and 40 Hz. Significant deflections of neuromagnetic fields were visually identified on the basis of RMS fields containing more than 10 sensors in the frontotemporal or temporooccipital regions. Locations and dipole moments of equivalent current dipoles were calculated every 2 msec for each selected time period by using the single equivalent dipole model. Only dipoles with a correlation value greater than 0.9 between measured and calculated field distributions were accepted. To confirm the calculated results, the same MEG time periods were analyzed using one of the following: current-density maps or LORETA (Curry; Neuroscan Labs, Sterling, VA).

The estimated dipoles were converted into three-dimensional MR images by identifying external anatomical fiduciary markers (nasion and left and right preauricular points).

Serial Changes in the Lexicosemantic MEG Studies

Preoperative MEG Findings. The patient and healthy volunteers could easily complete both tasks after a brief practice period. The mean reaction time and the percentage of successful task performance of the patient were approximately 800 msec and 92.3%, respectively, which were within normal range (Table 1). Figure 1 depicts the RMS fields of the preoperative MEG study *(thick black line)* with the kana-reading task in the bilateral frontotemporal and temporooccipital regions. Late deflections peaking at approximately 350 msec were observed in both of the left frontotemporal and temporooccipital regions. In the contralateral hemisphere (right frontotemporal and temporooccipital regions), however, early and short-duration RMS peaks were recorded approximately 250 msec after the stimulation. In all 15 healthy volunteers, the late deflections were predominantly observed in the left frontotemporal region rather than in the right hemisphere. On the other hand, in the temporooccipital region there was no late response in five volunteers (33.3%), left-side dominance in seven (46.7%), and right-side dominance in three (20%).

The figure discrimination task evoked only early deflections (within 300 msec) in both hemispheres with no later activation in the patient or any volunteer; therefore, we considered that the later responses in the left hemisphere might be strongly related to the lexicosemantic processes in letter perception on the basis of our preliminary results and previous reports.[7,9]

Figure 2 demonstrates the representative MEG sources in two healthy volunteers. The left hemispheric responses were mainly localized in the superior, middle temporal, and supramarginal gyri (mean number of dipoles 122.4). In contrast, in the right hemisphere there were far fewer numbers of estimated dipoles (mean number of dipoles 32.4). Concerning the location of the temporooccipital dipoles, it was not common for the left inferior temporal region to have predominantly more dipoles than the right side. There was no consistent dominance of temporooccipital dipoles between the hemispheres (Fig. 2).

In the patient, the estimated dipoles of the left frontotemporal response were mainly concentrated in the anterior portion of the left superior and middle temporal gyri (38 dipoles) (Fig. 3A and B). Additionally, 102 dipoles of the left temporooccipital responses were densely concentrated adjacent to the posterior border of the tumor in the fusiform gyrus (Fig. 3C and D), which was relatively strong compared with the control data. The right frontotemporal and temporooccipital responses of the patient were observed in the right supramarginal gyrus and in the fusiform gyrus, respectively. The 24 right-hemisphere dipoles did not reach even one third of that of the left hemisphere, indicating left-side dominance for the reading process in this patient. It was notable that all 102 dipoles in the left hemisphere were mainly located in the fusiform gyrus where the tumor invaded. The LORETA analysis demonstrated two clusters of stronger sources in the anterior portion of the left superior temporal gyrus and the left fusiform, as did the single equivalent dipole model. We reexamined the patient 4 days after the first examination to confirm that the lexicosemantic MEG should reveal the consistent results for preoperative functional mapping. The second MEG examination demonstrated that the left fusiform gyrus was extremely active with the letter-reading task, just as the first examination had.

Postoperative MEG Findings. On the 10th day after surgery, the patient could not complete the reading task due to severe dyslexia. He was, therefore, asked to look passively at the presented letters. The most significant change on the MEG study was that no significant responses were detected in the left hemisphere (Fig. 1).

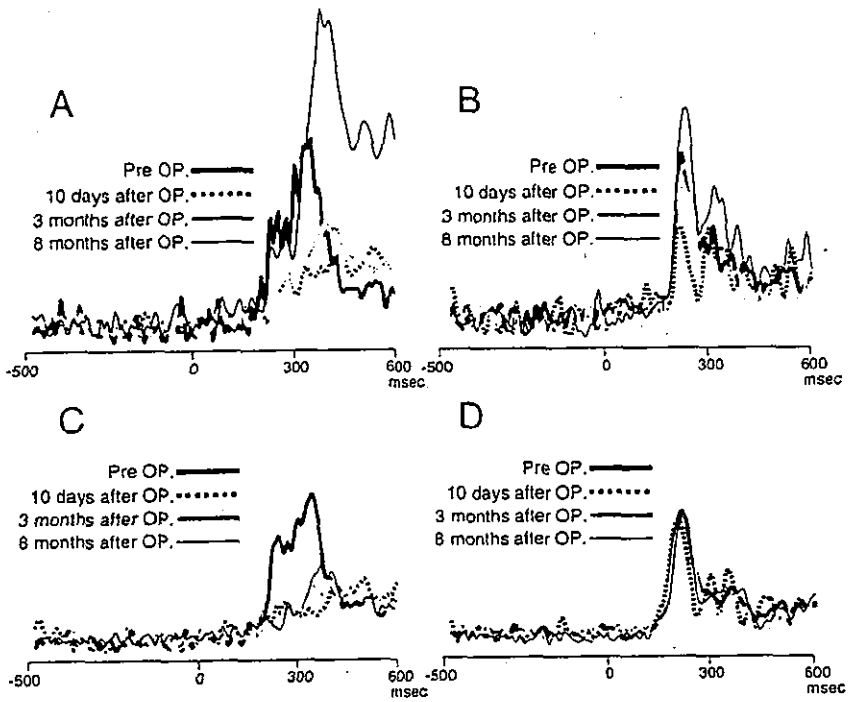


FIG. 1. Four RMS profiles of lexicosemantic MEG responses during the letter-reading task from the left (A) and right (B) frontotemporal regions and from the left (C) and right (D) temporooccipital regions. The left frontotemporal responses, which peaked at 400 msec, are markedly decreased in amplitude and the left temporooccipital responses became silent after the resection. Note that the RMS profile from the left frontotemporal region becomes approximately 1.5 times higher in amplitude 8 months after surgery, despite no changes in the bilateral temporooccipital regions.

Three months after surgery, the patient's reading skills had improved and he could slowly read kana character by character. His mean reaction time and rate of success were approximately 1320 msec and 63.8%, respectively, which remained worse than his performance preoperatively. Although small RMS deflections appeared in the left frontotemporal region, peaking at approximately 420 msec (later than the preoperative response), these responses were too small in amplitude to localize. There was no obvious deflection in the left temporooccipital region. In contrast to the left hemisphere, the RMS profiles detected in the right frontotemporal region were almost identical to the preoperative MEG studies.

Eight months after surgery, the patient had recovered notably from the dyslexia and could perform the reading task with some effort. His mean reaction time and rate of success were further improved. It is noteworthy that the amplitudes of the left frontotemporal responses were more than 1.5 times higher than those of the preoperative responses. Estimated dipoles of the left frontotemporal response were mainly concentrated in the anterior portion of the superior and middle temporal gyri (78 dipoles) (Fig. 4) and showed 56.4 nAm of the mean dipole moment, which was 1.5 times stronger than that of the preoperative response (36.2 nAm). The peak latency periods of the left frontotemporal responses, however, were still later than those measured preoperatively (at ~ 420 msec). The activities of the left temporooccipital region remained quiescent. The right frontotemporal region revealed a sharp RMS deflection with slightly high amplitudes, peaking at 250 msec after the stimuli. The right temporooccipital responses had been consistently peaking at approximately 250 msec with similar RMS amplitudes throughout the serial MEG investigations. The right hemisphere had 37 dipoles in the frontotemporal and temporooccipital regions.

Discussion

The radical resection of the mesial temporal glioma injured the left fusiform gyrus, where the lexicosemantic MEG dipoles were concentrated, and, as a result, caused severe dyslexia. The patient's impaired reading skills, however, were generally improved a year later. In the meantime the spared left frontotemporal region, which used to be one of the semantic centers, produced an overshot recovery of MEG responses. This finding indicates that MEG provides a noninvasive method of identifying and visualizing the lexicosemantic centers used in the reading process. It is a matter of course that the preoperative identification of eloquent cortices related to higher brain functions is beneficial for neurosurgical planning. Furthermore, it is scientifically important that the sequential recovery of MEG signals on repeated studies be observed along with the patient's clinical recovery from dyslexia.

Although it is well known that right-handed patients with left inferior temporal lesions suffer from impaired reading skills, we empirically know that dyslexia may not appear in 100% of these patients and that if it does, it sometimes is improved later. Researchers who have performed PET studies in healthy volunteers have reported that visually presented letters activate the bilateral superior temporal and posterior inferior temporal regions as well as the Broca area.[15,16]

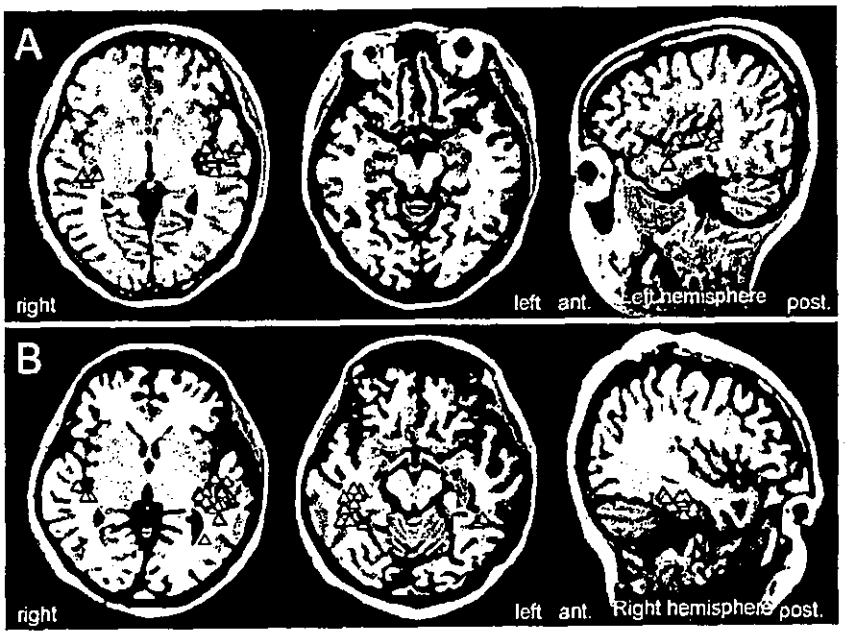


FIG. 2. Lexicosemantic MEG dipole distributions in two healthy volunteers (Volunteers A and B). Estimated dipoles of late deflections in the frontotemporal regions are predominantly concentrated in the left hemisphere (107 dipoles in Volunteer A and 90 in Volunteer B) compared with the right side (42 and 22 dipoles, respectively). The temporooccipital regions exhibit no late response (Volunteer A) and a right-side–dominant dipole distribution (45 dipoles in the right and four dipoles in the left hemispheres of Volunteer B).

Measurements of evoked potentials in patients with epilepsy have demonstrated responses at approximately 200 msec (N200) on the cortices of the bilateral temporal base, including the fusiform gyrus, after letter presentation.[1,10,11] The sole function of the fusiform gyrus can barely be investigated using cortical stimulation, fMR imaging or PET scanning, because of its anatomical characteristics (small size and deep location) and the surrounding vascular structures (the vein of Labbé and the basal veins of Rosenthal). The functional role and dominance of the fusiform gyrus, therefore, remain obscure.

Authors of recent MEG studies performed in healthy volunteers have found lexicosemantic activity in the fusiform gyrus and the left superior temporal gyrus at approximately 200 (early) and 400 (late) msec following letter presentation, respectively.[7,9] Authors of these studies have emphasized that the fusiform gyrus as well as the left superior temporal gyrus may principally contribute to reading processes. Although the temporooccipital regions of normal controls exhibited various dipole distributions, such as left-side dominance (46.7%), right-side dominance (20%), and no response (33.3%) in our preliminary study, strong activation was especially demonstrated in the left fusiform gyrus in our patient. On the basis of these results, the fusiform gyrus of the dominant hemisphere plays an important role for reading processes, but the functional dominance of this structure should be carefully investigated for each patient.

It is noteworthy that the patient's dyslexia remarkably improved until 8 months following resection of the fusiform gyrus in his dominant hemisphere. Previous PET and fMRI imaging studies have indicated a possibility of cortical reorganization in patients who display dramatic recoveries of motor functions.[3,4] These studies have demonstrated activations not only in the contralateral cortex, but also in the ipsilateral sensorimotor cortex and in other cortical regions, indicating the involvement of a widespread network in the recovery process.[2] In our case, the left temporooccipital region became silent on MEG following resec-

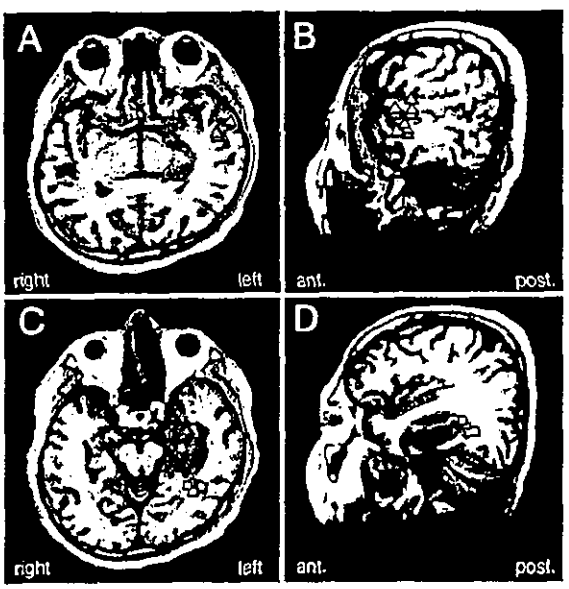


FIG. 3. Lexicosemantic MEG dipole locations in the left frontotemporal (A and B) and left temporooccipital regions (C and D) before surgery. The dipoles are concentrated in the anterior portion of the superior and middle temporal gyri (*white triangles*) and in the fusiform gyrus (*white squares*), which contains the tumor.

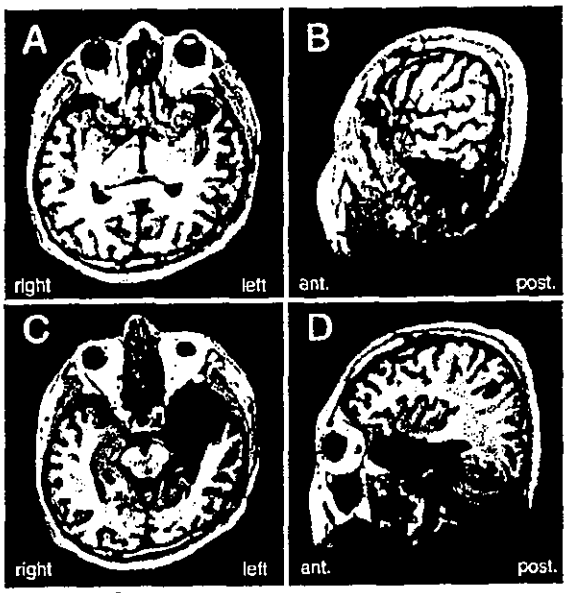


FIG. 4. Lexicosemantic MEG dipole locations in the left frontotemporal (A and B) and temporooccipital regions (C and D) 8 months after surgery. The left frontotemporal dipoles are located in the anterior portion of the superior and middle temporal gyri; however, there are no dipoles in the left temporooccipital region, including the fusiform gyrus.

tion, whereas the responses of the contralateral homologous (right) temporooccipital region constantly maintained the same RMS profiles. The left frontotemporal region showed a marked recovery in MEG amplitude, but the peak latency period did not completely return to its preoperative state. Although we observed no compensatory activity or reorganization in the ipsilateral temporooccipital region, the responses of the left frontotemporal region at 8 months after surgery became 1.5 times higher in amplitude than those before surgery. The patient experienced difficulty in reading letters after surgery and thus required more concentration to perform the reading task. One should consider, at least in part, that the spared left *frontotemporal region might* have contributed to the patient's recovery from dyslexia.

Our single equivalent dipole model provided a similar result to those of previous reports[7,8,13,17] and is helpful for understanding the process of language perception. Nevertheless, it is critical to consider the responsibility of multiple dipoles existing in the bilateral fusiform gyrus or in other regions, which may provide additional supplementary functions in the reading process. The LORETA is one of the currently available density maps that can potentially be used to analyze multiple sources in the electro- and magnetophysiological fields.[9] *It can separately localize* two or three active sources with different time courses, which the single dipole model fails to localize. Because LORETA and the single dipole approach yield the same results, the source *localization* of this study became more reliable. Furthermore, the resection of the fusiform gyrus that produced the active sources resulted in severe dyslexia.

Although preliminary, this case study demonstrates that *MEG performed using the kana reading task* can readily identify the semantic magnetic responses and provide a noninvasive means for analyzing functional brain structures relating to letter perception. To our knowledge, this is the first report in which a method has been introduced that can be used to predict a risk for postoperative dyslexia and monitor *functional recovery from the symptom*. This technique can be applied to analyze other semantic processes and will be a useful tool in the elucidation of the pathophysiology of aphasia, dysphasia, and dyslexia.

References

1. Allison T, McCarthy G, Nobre A, et al: Human extrastriate visual cortex and the perception of faces, words, numbers, and colors. **Cereb Cortex 4:**544–554, 1994
2. Chollet F, DiPiero V, Wise RJS, et al: The functional anatomy of motor recovery after stroke in humans: a study with positron emission tomography. **Ann Neurol 29:**63–71, 1991
3. Cohen L, Lehericy S, Chochon F, et al: Language-specific tuning of visual cortex? Functional properties of the Visual Word Form Area. **Brain 125:**1054–1069, 2002
4. Feydy A, Carlier R, Roby-Brami A, et al: Longitudinal study of motor recovery after stroke: recruitment and focusing of brain activation. **Stroke 33:**1610–1617, 2002
5. Geschwind N: The organization of language and the brain. **Science 170:**940–944, 1970
6. Iwata M: Neural mechanism of *reading and writing in the* Japanese language. **Funct Neurol 1:**43–52, 1986
7. Kamada K, Kober H, Saguer M, et al: Responses to silent Kanji *reading of the native Japanese and German in task* subtraction magnetoencephalography. **Brain Res Cogn Brain Res 7:**89–98, 1998
8. Kuriki S, Hirata Y, Fujimaki N, et al: Magnetoencephalographic study on the cerebral neural activities related to the processing of visually presented characters. **Brain Res Cogn Brain Res 4:** 185–199, 1996
9. Mulert C, Gallinat J, Pascual-Marqui R, et al: Reduced event-related current density in the anterior cingulate cortex in schizophrenia. **Neuroimage 13:**589–600, 2001
10. Nobre AC, McCarthy G: Language-related ERPs: scalp distributions and modulation by word type and semantic priming. **J Cogn Neurosci 6:**233–255, 1994
11. Nobre C, Allison T, McCarthy G: Word recognition in the human inferior temporal lobe. **Nature 372:**260–263, 1994
12. Oldfield RC: The assessment and analysis of handedness: the Edinburgh inventory. **Neuropsychologia 9:**97–113, 1971
13. Petersen SE, Fox PT, Posner MI, et al: Positron emission tomographic studies of the cortical anatomy of single-word processing. **Nature 331:**585–589, 1988
14. Sakurai Y, Momose T, Iwata M, et al: Semantic process in kana word reading: activation studies with positron emission tomography. **NeuroReport 4:**327–330, 1993
15. Salmelin R, Service E, Kiesila P, et al: Impaired visual word processing in dyslexia revealed with magnetoencephalography. **Ann Neurol 40:**157–162, 1996
16. Simos PG, Breier JI, Fletcher JM, et al: Brain activation profiles in dyslexic children during non-word reading: a magnetic source imaging study. **Neurosci Lett 290:**61–65, 2000

Manuscript received December 12, 2002.
Accepted in final form February 9, 2004.

Address reprint requests to: Kyousuke Kamada, M.D., Department of Neurosurgery, Graduate School of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo, 113-8655, Japan. email: kamady-k@umin.ac.jp.