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Imaging Correlates of Molecular Signatures in Oligodendrogliomas

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ABSTRACT

Molecular subsets of oligodendroglioma behave in biologically distinct ways. Their locations in the brain, rates of growth, and responses to therapy differ with their genotypes. Retrospectively, we inquired whether allelic loss of chromosomal arms *1p* and *19q*, an early molecular event and favorable prognostic marker in oligodendrogliomas, were reflected in their appearance on magnetic resonance imaging. Loss of *1p* and *19q* was associated with an indistinct border on T_1 images and mixed intensity signal on T_1 and T_2. Loss of *1p* and *19q* was also associated with paramagnetic susceptibility effect and with calcification, a common histopathological finding in oligodendrogliomas. These data encourage prospective evaluation of molecular alterations and magnetic resonance imaging characteristics of glial neoplasms.

INTRODUCTION

Oligodendrogliomas were the first of the malignant gliomas shown to be responsive to chemotherapy and the first for which specific molecular predictors of chemotherapeutic response and survival were identified (1–4). In anaplastic oligodendrogliomas, response to chemotherapy, duration of response to chemotherapy, progression-free survival after radiotherapy, and overall survival have been associated with allelic loss of chromosomal arm *1p* (1–8). Moreover, loss of *1p* is highly associated with loss of *19q*, together constituting the earliest known molecular change in 50–70% of such neoplasms (9). Combined loss of *1p* and *19q* appears to define a treatment-sensitive malignant glioma, one which may sometimes be curable with current therapies (7). Indeed, diagnostic molecular testing may soon guide the management of patients with low- and high-grade oligodendrogliomas.

Like genetics, imaging is also revolutionizing neuro-oncology. Today, the detection, surgical management, radiation planning, chemotherapeutic assessment, and follow-up evaluation of patients with brain tumor are highly dependent on magnetic resonance imaging (MRI). Tissue sampling is needed for precise diagnosis, but the type of brain tumor can often be correctly inferred from the clinical context and MRI. Moreover, in adults with cerebral gliomas, enhancement with gadolinium is usually indicative of a high-grade anaplastic tumor, with ring-enhancement being characteristic of the most malignant of these, glioblastoma. To further explore the potential of MRI to provide diagnostic information, we tested the hypothesis that *1p* and *19q* allelic loss in an oligodendroglioma might be associated with specific imaging characteristics.

MATERIALS AND METHODS

Patient Selection. After institutional review board approval, cases for study were culled from a database of patients with oligodendrogliomas. The eligibility criteria included the following: (*a*) a newly diagnosed, untreated oligodendroglioma; (*b*) a preoperative MRI scan; and (*c*) data on *1p* and *19q* status.

Imaging Analysis. MRI scans were assessed by a neuroradiologist (D. H. L.) and two neurosurgeons (J. F. M., E. K.) blinded to the genetic alterations in the tumors. Only T_1-weighted, T_1 post-gadolinium, and T_2-weighted images were consistently available and reviewed. The following image characteristics were evaluated qualitatively: (*a*) sharp *versus* indistinct tumor border; (*b*) homogeneous *versus* heterogeneous tumor signal intensity (*i.e.*, hypointense on T_1 and hyperintense on T_2 *versus* mixed intensity signals on T_1 and T_2); (*c*) paramagnetic susceptibility effect, present *versus* absent [*i.e.*, shortening on T_1 and T_2 (10, 11)]; (*d*) contrast enhancement, present *versus* absent; and (*e*) percent cortical involvement by tumor (*i.e.*, maximum cortical extent divided by maximum tumor diameter). The images were scored by consensus. Pathology slides and reports on each case were re-reviewed by a neuropathologist (D. N. L.) to ascertain whether calcification or hemorrhage had been present in the tumor at the time of initial diagnosis.

Molecular Studies. Allelic status was assessed by loss of heterozygosity assays in constitutional/tumor DNA pairs using microsatellite markers on *1p36* (*D1S2734*, *D1S199*, *D1S508*) and *19q13* (*D19S219*, *D19S112*, *D19S412*, *D19S596*; Ref. 3). Tumor DNA was extracted from microdissected, formalin-fixed, paraffin-embedded sections and constitutional DNA from blood leukocytes or paraffin sections of adjacent uninvolved brain (12).

Statistical Considerations. Fisher's exact test was used to assess the significance of associations between *1p* loss and *19q* loss and imaging features (with % cortical involvement

Received 2/3/04; revised 3/27/04; accepted 4/7/04.
Grant support: Canadian Institutes of Health Research Grant 37849, NIH Grant CA57683, and the Alberta Cancer Foundation.
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dichotomized at its median). The overall type 1 error of 0.05 was controlled using a bootstrap resampling method with 10^5 samples (13), as implemented in SAS, PROC MULTTEST (SAS Institute Inc., Cary, NC). The Wilcoxon rank-sum test was used to assess the association between measured cortical involvement and genetic features. The adjusted *P* values are reported.

RESULTS AND DISCUSSION

Forty sets of scans were evaluated on 21 men and 19 women, ages 24–82 years (median, 40 years). The histopathological, molecular genetic, and imaging features are summarized in Table 1. There were 12 WHO grade II tumors, 13 grade II-III tumors, and 15 grade III oligodendrogliomas. Oligoastrocytomas were excluded from this study. Loss of chromosomal arm *1p* was detected in 21 tumors, loss of *19q* was observed in 23 (three were non-informative), and combined loss of *1p* and *19q* was noted in 18. Both alleles, *1p* and *19q*, were intact in 13. The tumor border was indistinct on T_1-weighted images in 28 cases and indistinct on T_2 images in 23. In no instance was the border sharp on T_1 and indistinct on T_2. The tumor signal intensity was heterogeneous on T_1 and T_2 in 28 cases, and susceptibility effect was present in 15. Twenty tumors displayed evidence of contrast enhancement. Calcification was present in the tumor sections or described in the pathology reports in 12. There was no evidence of hemosiderin deposition in any case.

A significant association was observed between tumor genotype and tumor border on T_1 images. Oligodendrogliomas with *1p* and *19q* loss were significantly more likely to have an indistinct border, whereas a sharp border between tumor and adjacent brain was characteristic of those with intact *1p* and *19q* alleles (Fig. 1). Of 26 with an indistinct border and full genetic data, 17 had *1p* and *19q* loss, and of 12 with a sharp border only one had *1p* and *19q* loss ($P = 0.005$). The association of tumor genotype with an MRI characteristic likely to be indicative of invasiveness is consistent with an earlier study in which all bi-hemispheric oligodendrogliomas harbored deletions of *1p* and *19q* (14). The biological basis of this association is unclear, although one potential explanation posits that *1p* and *19q* allelic loss confers an invasive phenotype. Indeed, in a recent microarray analysis, oligodendrogliomas with *1p* and *19q* loss had a gene expression profile similar to that of normal brain (15), which might occur if normal brain tissue was trapped within an invasive tumor. Alternatively, susceptibility to transformation by *1p* and *19q* loss might occur preferentially in cells that are intrinsically motile. In this scenario, the cell of origin determines the invasive character of the tumor, the latter reflected in the MRI. In either case, the fact that oligodendrogliomas with *1p* and *19q* loss may be especially invasive could have therapeutic implications. Successful treatment for oligodendrogliomas harboring *1p* and *19q* allelic loss might require drugs that safely penetrate the entire central nervous system.

An association was also observed between genotype and tumor signal intensity on T_1 and T_2 images. Oligodendrogliomas with *1p* and *19q* loss were more likely to display mixed signal intensity, whereas a uniform signal on T_1 and T_2 images was more characteristic of those with intact alleles (Fig. 1). Of 26 tumors with mixed signal intensity, 16 had *1p* and *19q* loss, and of 12 with homogeneous intensity only two had *1p* and *19q* loss ($P = 0.047$).

In addition, there was a significant association between tumor genotype and paramagnetic susceptibility effect. Oligodendrogliomas with *1p* and *19q* loss were more likely to display susceptibility change, whereas the absence of susceptibility was characteristic of tumors with intact alleles (Fig. 1). Of 15 tumors with susceptibility present, 13 had *1p* and *19q* loss, and of 23 without susceptibility 6 had *1p* and *19q* loss ($P = 0.007$). Calcification and tumor-associated hemorrhage are histopathological features of oligodendroglioma (16). Because both features might contribute to signal heterogeneity and susceptibility effect, we re-reviewed the pathology slides and reports on all cases. Hemosiderin was not detected. There was, however, a significant association between intratumoral calcification and the presence of paramagnetic effects ($P = 0.03$). There was also a significant association between calcification and *1p* and *19q* loss. Of 12 tumors with calcification, 10 had *1p* and *19q* loss, and of 25 with no apparent calcification 7 had *1p* and *19q* loss ($P = 0.007$).

In the computed tomography era of brain tumor diagnosis, calcification in a supratentorial intra-axial tumor was highly suggestive of an oligodendroglioma. Calcification was a common radiographic finding in low-grade oligodendrogliomas and in anaplastic tumors that had evolved from a lower grade lesion, a natural history profile associated with *1p* and *19q* loss (17). Indeed, in our series of cases diagnosed by computed tomography, 17 of 19 calcified tumors had *1p* and *19q* loss, one had *1p* loss (*19q*, non-informative), and one had intact alleles.[5] It is tempting to speculate that calcification is a biological effect downstream of *1p* and *19q* loss, that calcification is reflected by paramagnetic effects on MRI, and therefore, that paramagnetic susceptibility changes constitute a marker of *1p* and *19q* loss in

Table 1 Clinical, pathological, genetic, and imaging features

Number of patients	40
Age at diagnosis, median (range)	40 (24–82)
Sex (M/F)	21/19
Pathology	
Grade II	12/40
Grade II-III	13/40
Grade III	15/40
Calcification detected	12/37 [b]
Genetics	
1p LOH [a]	21/40
19q LOH	23/37 [b]
1p and *19q* LOH	18/38 [b]
1p and *19q* intact	13/37 [b]
Imaging	
Contrast enhancement present	20/37 [b]
Indistinct tumor border (T_1)	28/40
Indistinct tumor border (T_2)	23/40
Heterogeneous tumor signal (T_1-T_2)	28/40
Susceptibility effect present	15/40
Cortex involvement, median (range)	83% (39–100%)

[a] LOH, loss of heterozygosity.
[b] Denominator <40 denotes missing or ambiguous data.

[5] Unpublished observations.

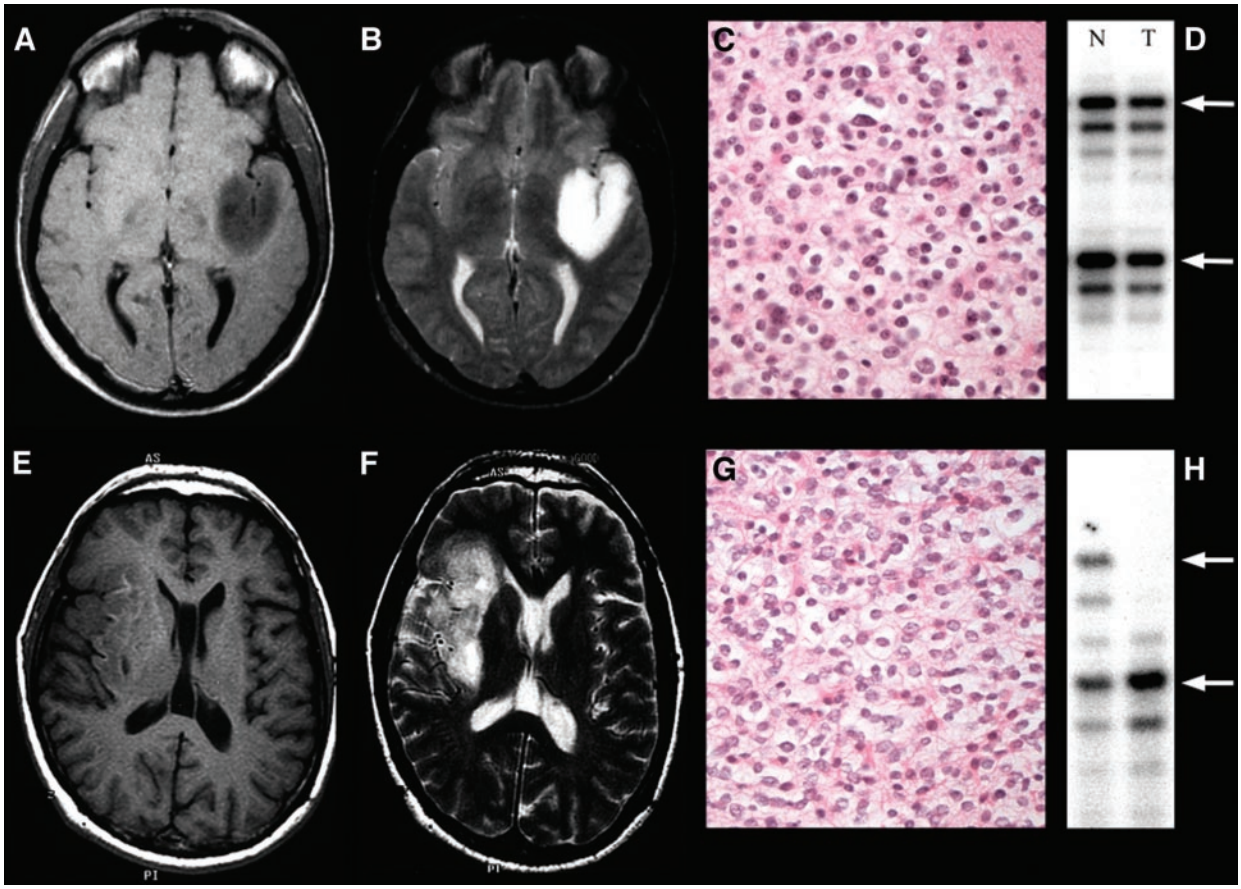

Fig. 1 The *top row* shows a T_1-weighted image (*A*), a T_2 image (*B*), and a photomicrograph (*C*) of an oligodendroglioma with intact *1p* and *19q* alleles. The tumor has a sharp border on T_1, no evidence of susceptibility effect, homogeneous signal intensity on T_2, and retained alleles at *D1S2734* on chromosome *1p* [*D, arrows* point to parental alleles that are present in both normal (N) and tumor (T) DNAs]. The *bottom row* shows a T_1-weighted image (*E*), a T_2 image (*F*), and a photomicrograph (*G*) of an oligodendroglioma with *1p* and *19q* loss. The tumor has an indistinct border on T_1, susceptibility effect (bright curvilinear markings) on T_1, heterogeneous signal intensity on T_2, and loss of heterozygosity at *D1S508* on *1p* [*H, arrows* point to the loss of one of the parental alleles in the tumor (T) DNA that is retained in the normal (N) DNA sample].

oligodendrogliomas. However, because calcium is a nonparamagnetic element, the relationship between intratumoral calcification and susceptibility effects is likely indirect. Perhaps, in addition to often being calcified, oligodendrogliomas with *1p* and *19q* loss contain paramagnetic elements. Certainly such findings encourage additional study.

The association of *1p* and *19q* loss with an indistinct border, mixed signal intensity, and susceptibility was also true for *1p* loss, with or without *19q* loss (Table 2). There was no statistical association between *19q* loss and any imaging parameter. A similar pattern was seen in an earlier study in which radiographic response to chemotherapy was tightly linked to *1p* and *19q* loss and also to *1p* loss but not to *19q* loss (3). Loss of *19q*, a common finding in high-grade gliomas, appears to be associated with a more favorable prognosis (18). However, *19q* loss *per se* is less characteristic of oligodendroglial histopathology than is *1p* loss.

Clinical MRI, as performed here, will not replace tissue-based diagnosis. However, the possibility that molecular alterations in gliomas might be detected by quantitative imaging, novel sequences (19), or biochemical surrogates (20) is strongly encouraged by our findings. Furthermore, the association of *1p* and *19q* loss with specific imaging and histopathological features may provide clues to the function of genes on chromosomes *1p* and *19q* that appear critical to the genesis of oligodendrogliomas. A note of caution is warranted. The aforementioned associations require confirmation in a larger

Table 2 *P* values for associations of imaging with *1p* and *19q* allelic loss

Imaging feature	*P* value		
	1p and *19q* loss	*1p* loss	*19q* loss
Contrast enhancement	0.288	0.336	0.921
Indistinct border–T_1	0.005	0.001	0.214
Indistinct border–T_2	0.092	0.088	0.944
Heterogeneous signal	0.047	0.017	0.992
Susceptibility effect	0.007	0.035	0.061
Cortical involvement	0.961	0.628	0.999

P values are based on Fisher's exact test and adjusted for multiple comparisons.

prospective study that includes a more comprehensive set of MRI sequences and quantitative image analysis.

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