The Use of Magnetic Resonance Imaging to Noninvasively Detect Genetic Signatures in Oligodendroglioma

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Abstract

Background: Some patients with low-grade glioma have extraordinarily long survival times; current, early treatment does not prolong their lives. For this reason, therapies that sometimes have neurologic side effects are often deferred intentionally.

Methods: In a study of oligodendrogliomas, we used a quantitative method of MR analysis based on the S-transform to investigate whether codeletion of chromosomes 1p and 19q, a marker of good prognosis, could be predicted accurately by measuring image texture.

Results: Differences in texture were seen between tumors with codeletion of chromosomes 1p and 19q and those with intact 1p and 19q alleles on contrast-enhanced T1-weighted and T2-weighted MR images. Quantitative MR texture on T2 images predicted codeletion of chromosomes 1p and 19q with high sensitivity and specificity.

Conclusions: This new method of MR image interpretation may have the potential to augment the diagnostic assessment of patients with suspected low-grade glioma.

In neuro-oncology, a sensitive and specific imaging-based molecular test that reliably detects important genetic signatures in gliomas would be a helpful diagnostic adjunct. Such a capability would be especially useful in the case of a young adult with a first seizure, a normal neurologic examination, and a probable low-grade glioma on magnetic resonance (MR) imaging, a common clinical scenario. Many such patients have slow-growing oligodendrogliomas with codeletion of chromosomes 1p and 19q, a favorable genetic feature. Understandably, neurologically intact patients destined to have long survival, and without compelling reasons for early interventions that do not prolong length of life, might wish to defer therapeutic maneuvers that could have neurologic side effects, especially if a genetic signature associated with excellent prognosis, like codeletion of 1p and 19q, could be detected noninvasively. The ability to ascertain on an imaging study that a probable low-grade glioma has a favorable genetic profile and will behave in an indolent manner would give patients and clinicians greater confidence and flexibility in designing a disease management strategy. Furthermore, an imaging-based molecular test would complement the results of a stereotactic biopsy; up to 5% of tumor biopsies are not definitive because the volume of tissue retrieved is too small for histologic or genetic evaluation.

MR imaging is a staple of brain tumor diagnosis. Although MR cannot yet resolve features on the scale of chromosomes, several advanced techniques can detect clinically relevant molecular processes in glioma. Spectroscopy can measure important tumor metabolites (1) and perfusion-weighted MR can detect angiogenesis (2); both correlate with glioma grade and prognosis. In this study, we use a quantitative method of image analysis developed by our group, S-transform-based texture analysis, to test the hypothesis that 1p and 19q codeletion in oligodendrogliomas can be detected using MR (3). This study builds on earlier work in which we used standard visual analysis of MR images to describe signal characteristics associated with 1p/19q loss (4).

The texture of a feature in an MR image can be defined as a local characteristic pattern of visual intensities. Aspects of texture can be quantified by assessing the local spatial-frequency content: strong lower frequencies appear as homogeneous smooth regions, whereas strong higher frequencies are...
seen as heterogeneous detailed regions. Quantification of texture was developed for satellite imaging applications and is now being applied to the analysis of medical images. One major class of texture quantification examines the Fourier transform of a region in an image. Peaks in the spectrum reflect the orientation and period of textural patterns. Measurements that are insensitive to orientation can be obtained by transforming the two-dimensional Cartesian Fourier spectrum to polar coordinates: the angle-coordinate describes feature orientation whereas the radius-coordinate describes an orientation-independent frequency spectrum (5). The S-transform is a localized Fourier transform that provides local frequency spectra for each pixel in an image (6, 7). Performing the orientation-insensitive spectral analysis procedure on each local spectrum produced by the S-transform allows a pixel-by-pixel examination of image texture.

**Materials and Methods**

**Patient and image selection.** Texture analysis was done retrospectively on the initial diagnostic MR scans (1.5 T; GE Healthcare) of 55 cases from a single neurosurgical and cancer center (33 males, 22 females; median age, 43 y) that met the following criteria: (a) a biopsy-proven low-grade oligodendroglioma or mixed oligoastrocytoma (oligodendroglioma-dominant; >75% oligodendroglioma); (b) known molecular genetic characteristics, either codeletion of chromosomes 1p and 19q \( (n = 31; 56\%) \) or intact 1p and 19q alleles \( (n = 24; 44\%) \); and (c) available preoperative MR images that included at least two of the following sequences: contrast enhanced T1-weighted, T2-weighted, or FLAIR sequences. The imaging parameters were, for T1-weighted, median repetition time, 539 ms; median echo time, 9 ms; for T2-weighted, median repetition time, 4967 ms; median echo time, 103 ms; and for FLAIR-weighted, median repetition time, 9004 ms; median echo time, 171 ms; median inversion time, 171 ms. By design, cases with chromosome 1p deletion only \( (n = 0) \), chromosome 19q deletion only \( (n = 2) \), or low-quality preoperative MR images \( (n = 2) \) were excluded from the analysis. Genotyping was accomplished with loss of heterozygosity analysis \( (n = 43) \) or fluorescence in situ hybridization \( (n = 12) \). The median time from MR imaging to surgical resection yielding tumor tissue for genotyping was 58 d.

**Texture analysis.** Using the S-transform, we determined the frequency spectrum for each pixel in a region of interest (ROI) in the MR image of each brain tumor. By averaging each frequency component over all directions, a set of one-dimensional spectra was obtained for each tumor. These sets of spectra were subjected to statistical comparisons and receiver operator characteristic (ROC) analysis (8). A two-sample Hotelling’s \( T^2 \) test was used to examine
entire spectra from each MR contrast for significant differences between tumors with intact and deleted 1p/19q. For contrasts where differences were detected, Bonferroni corrected 95% confidence intervals were calculated to identify particular frequencies that differed significantly between tumor genotypes, and \( P \) values were calculated with Student’s \( t \) tests for those frequencies. Sensitivity, specificity, positive and negative predictive values, likelihood ratios, and ROC curves were also calculated for each frequency. The ROI for texture analysis for each of the 55 cases consisted of the largest rectangular area within the visible tumor on each slice of the image using the FLAIR sequence to identify the tumor; the T2-weighted sequence was used to define the ROI in one case.

Likelihood ratios were used as a normalized scale to combine results from several of the most discriminatory frequencies to form a multifrequency classifier. A leave-one-out analysis was then conducted on this composite measure. For each round of leave-one-out analysis, the five frequencies with the highest combined sensitivity and specificity were identified and used to classify the excluded patient data. Because echo time and repetition time were not constant, an ANOVA was done on T1- and T2-weighted images to control for the effect of imaging parameter variation on spectral differences. Spectra from FLAIR images did not show significant differences in relation to genotype and were not included in the ANOVA analysis.

**Results**

Standard MR images of the oligodendrogliomas in this series often looked similar (Fig. 1) but significant differences in the amplitudes of intermediate frequencies were detected on both contrast-enhanced T1-weighted and T2-weighted images, using S-transform–based texture analysis, with codeleted tumors
showing increased spectral power (Fig. 2). No significant differences in image texture were detected between codeleted and intact cases on FLAIR images, however. T2-weighted MR images showed both the greatest portion of significant spectrum (0.058-0.458 cycles/mm; P < 0.05) and the most significant individual frequency (0.142 cycles/mm; P < 0.00001; Fig. 2; Table 1). Considered as a whole, the spectra from T2-weighted images differed significantly between intact and deleted tumors [two-sample Hotelling’s T2 = 134; F = 1.69; degrees of freedom (df), 32,21; P < 0.0006]. These results are consistent with the qualitative data (i.e., visual inspection of MR images) that emerged from our earlier study (4), and once again suggest that oligodendrogliomas with codeletion of chromosomes 1p and 19q have recognizably different MR imaging characteristics than oligodendrogliomas with intact 1p and 19q alleles.

To evaluate the performance of S-transform–based texture analysis as a possible predictive test of tumor genotype for individual cases and to identify potential ideal thresholds for 1p and 19q genotyping in a future validation set of prospective cases of unknown histology, ROC curves were generated for each frequency in the local spectrum. This assessment confirmed that T2-weighted images may have the best capacity to discriminate codeleted tumors from those with other genotypes. Spectral frequencies between 0.171 and 0.350 cycles/mm had similar ROC curves, all with areas >0.84. The best frequencies for distinguishing codeleted tumors from intact cases in this initial series were between 0.142 and 0.158 cycles/mm in T2 images, which had areas under the ROC curve of 0.93, sensitivity of 0.93, and specificity of 0.92 (Fig. 2D). Combining multiple frequencies improved the classification accuracy slightly. Using the top five frequencies in a leave-one-out analysis yielded area under the ROC curve = 0.94 ± 0.00077, sensitivity = 0.93 ± 0.00061, and specificity = 0.96 ± 0.00048, where the error term is the 95% confidence interval. Echo time and repetition time were not significantly associated with differences in frequency spectra on T2 images (F = 0.81; df, 1.49; P > 0.37 for echo time, and F = 0.81; df, 1.49; P > 0.37 for repetition time) but the status of chromosomes 1p and 19q was significantly associated (F = 3.8 × 1012; df, 1.49; P < 0.0001).

To explore which method of image assessment might have greater potential to predict oligodendroglioma genotype, S-transform–based texture analysis as described here or visual interpretation by blinded experts, a neuro-radiologist and a neurologist blindly viewed each case in the current series and rated the signal characteristics of each tumor in an effort to predict oligodendroglioma genotype as described by Megyesi et al. (4). The two clinicians achieved a sensitivity of 0.70 and a specificity of 0.63 for genotype prediction (Table 2). In addition, the results of Megyesi et al. (4) were reanalyzed to estimate the sensitivity and specificity of genotype prediction. Using T2-weighted signal homogeneity to classify these previously reported oligodendroglioma cases (n = 40), neuro-radiologists achieved a sensitivity of 0.67 and a specificity of 0.75 for detection of codeletion of chromosomes 1p and 19q by visual interpretation of the MR images (Table 2).

**Table 1. Frequencies with the highest area under ROC curve for each MR contrast**

<table>
<thead>
<tr>
<th>Sequence</th>
<th>Frequency</th>
<th>P</th>
<th>AROC</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1 + contrast</td>
<td>0.133</td>
<td>&lt;0.04</td>
<td>0.748</td>
<td>0.733</td>
<td>0.750</td>
</tr>
<tr>
<td>FLAIR</td>
<td>0.092</td>
<td>&lt;0.12</td>
<td>0.667</td>
<td>0.774</td>
<td>0.591</td>
</tr>
<tr>
<td>T2</td>
<td>0.142</td>
<td>&lt;0.00001</td>
<td>0.924</td>
<td>0.933</td>
<td>0.917</td>
</tr>
<tr>
<td>T2 multifrequency</td>
<td>Best 5</td>
<td>N/A</td>
<td>0.943 ± 0.0008</td>
<td>0.933 ± 0.0006</td>
<td>0.958 ± 0.0005</td>
</tr>
</tbody>
</table>

NOTE: Spectra from T2-weighted images both have the most significant differences and are best at detecting 1p/19q codeletion. The last row indicates the results of combining the five most discriminatory frequencies. Error values are provided by a leave-one-out analysis and indicate the 95% confidence interval. Abbreviation: AROC, area under the ROC curve.

**Table 2. Methods of detection of 1p and 19q codeletion from MR images**

<table>
<thead>
<tr>
<th>Method</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
<th>LR+</th>
<th>LR-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radiologist (Megyesi; ref. 4)</td>
<td>0.67</td>
<td>0.75</td>
<td>0.89</td>
<td>0.43</td>
<td>2.67</td>
<td>0.444</td>
</tr>
<tr>
<td>Radiologist (current study)</td>
<td>0.70</td>
<td>0.63</td>
<td>0.70</td>
<td>0.63</td>
<td>1.87</td>
<td>0.480</td>
</tr>
<tr>
<td>Texture analysis (current study)</td>
<td>0.93 ± 0.00061</td>
<td>0.96 ± 0.00048</td>
<td>0.97 ± 0.00033</td>
<td>0.92 ± 0.00089</td>
<td>22.4 ± 0.25</td>
<td>0.0696 ± 0.00064</td>
</tr>
</tbody>
</table>

NOTE: The performance of human observers in the current study (row 2) is similar to that of observers in our previous study, which used a different data set (row 1). The quantitative texture analysis method used in this article (row 3) performed much better (93% sensitivity; 96% specificity) and at a level of accuracy that is clinically relevant. Error values indicate the 95% confidence interval obtained from a leave-one-out analysis. Abbreviations: PPV and NPV, positive and negative predictive values, respectively. LR+ and LR-, positive and negative likelihood ratios, respectively.

Discussion

The first example of the practical application of molecular discovery to neuro-oncology was the recognition of the clinical relevance of coincident allelic loss of chromosomal arms 1p and 19q in oligodendrogliomas (9, 10). Codeletion of 1p and 19q, likely a manifestation of a recurring translocation (11, 12), is highly associated with oligodendroglial histopathology. In anaplastic oligodendrogliomas, codeletion is a marker of radiographic response to chemotherapy, durable tumor control.
after radiotherapy, and long overall survival (10, 13–15), and in low-grade tumors, predicts a very favorable natural history. Indeed, several studies have suggested that "genetics" may be a better predictor of key outcomes than histologic classification (16). Earlier work by our group raised the possibility that imaging features and tumor genotype might be related in oligodendrogliomas. In a retrospective analysis, peripheral tumor location and bihemispherical growth pattern were significantly associated with codeletion of chromosomes 1p and 19q (17). In a follow-up study, heterogeneous signal intensity on T2-weighted images and indistinct tumor border on T1-weighted images (i.e., differences in MR image texture) were associated with codeletion of 1p and 19q (4). Others have also found that MR features may be associated with tumor genotype. MR characteristics including visual texture have been associated with 1p and 19q loss in low-grade gliomas and EGFR amplification in glioblastomas (18, 19). Further, 1p and 19q codeletion has been associated with cerebral blood volume perfusion measurements (20) and diffusion-weighted imaging features (21) on MR and also with contrast uptake in nuclear images (22). These findings raise the possibility that clinically relevant molecular signatures in gliomas, particularly oligodendrogliomas, might be detected noninvasively using MR.

We investigated the possibility that a new quantitative method of MR image analysis based on the S-transform might be used to distinguish oligodendrogliomas harboring the 1p and 19q codeletion from those with other genetic alterations. Qualitative differences in the MR image texture of oligodendrogliomas are of interest (4), but visual methods of scan interpretation are subject to intraobserver variation and not generally useful as a diagnostic test. In the study reported here, spectral frequencies in the intermediate range were the most informative with respect to distinguishing codeletion from intact 1p and 19q alleles in oligodendrogliomas. Low spatial frequencies on T2-weighted and T1-weighted images were not useful discriminators of texture and genotype, perhaps because low frequencies are sensitive to features of an image that are large, such as tumor size or brain anatomy. Similarly, high spatial frequencies were not good discriminators of texture and genotype, perhaps because high frequencies are of low amplitude, often obscured by the noise of MR acquisition. Features of an MR image giving a heterogeneous or indistinct appearance when viewed with the naked eye, such as described by Megyesi et al. (4), are likely best captured by spectral frequencies in the intermediate range.

The mammalian visual cortex houses abundant image processing capability, including neurons that are sensitive to edges of particular orientation (23). Studies examining striate receptive fields in two dimensions have revealed neurons that are sensitive to changes in intensity with particular spatial frequencies and orientations (24). The behavior of these fields belongs to a class of linear spatial filters analogous to Gabor filters (25). A Gabor filter is a sinusoid modulated by a Gaussian, which produces an ideal localized measure of spatial frequency. A set of Gabor filters of particular frequencies and Gaussian widths can be used to calculate local frequency content for each region in an image. The S-transform (6) performs this operation, producing a local frequency spectrum for each pixel in an image. For a two-dimensional image, each of the local spectra is also two-dimensional, including information on both frequency amplitude and orientation. Here, we discard the directional information to achieve a texture measure that is insensitive to pattern orientation and seemingly able to distinguish between tumor genotypes.

Although the S-transform–based technique can be used with a ROI of any shape, including an arbitrary shaped ROI encompassing the entire tumor, we chose to use simple rectangular ROIs. For eventual clinical application, these may be faster and easier to draw than tracings of the tumor border, particularly in the case of tumors with indistinct transitions to normal-appearing brain tissue. In addition, because the S-transform is a windowed analysis technique, information from the area around a selected pixel will be included in the spectrum for that pixel. S-transform texture analysis of a ROI that attempts to trace the tumor border will therefore include information from normal-appearing brain tissue outside the region selected to be tumor tissue. For this study, our goal was to obtain a large, representative sample of the tumor that was free of edge effects. Here, smaller anatomic features were included in the ROI, but large cystic or fluid-filled areas were excluded.

S-transform–based texture analysis may have the potential to recognize 1p and 19q codeletion in MR images of low-grade gliomas. Before such a technique is ready to be used clinically, however, further evaluation is needed. Most importantly, this retrospective analysis does not mimic real life. All of the cases in this study were known to be oligodendrogliomas, whereas in real clinical situations, patients present with neurologic symptoms and have an MR examination that reveals a probable low-grade glioma, of which there are several different types. To be truly useful clinically, texture analysis will need to be able to detect codeletion or other important molecular alterations in gliomas with a high degree of accuracy and in a setting where the histologic diagnosis is unknown. That said, this technique may hold substantial promise.

Quantitative MR texture of T2 sequences predicted codeletion of 1p and 19q with a sensitivity of 93% and a specificity of 96% using images that were similar overall, but acquired with variable scanning parameters. This is encouraging because the ideal noninvasive predictive test would transcend minor differences in image acquisition. We used a single spatial frequency component to probe genotype prediction in oligodendrogliomas and also used a multifrequency method (five-best). Both were highly accurate and warrant further assessment. In the future, using multiple frequencies to predict genotype may reduce noise and provide a more robust noninvasive test. As it stands, we have shown that oligodendrogliomas with codeletion of chromosomes 1p and 19q have different MR image textures than those with intact alleles. Although the biological meaning of this difference in tumor texture remains unknown, it may nonetheless afford a method for detecting 1p and 19q codeletion in low-grade gliomas that is noninvasive and uses data from routinely acquired MR images. If perfected, such a technique would use a widely available imaging technology and approach the “gold standard” for genotyping, which is tumor biopsy followed by molecular genetic testing, but without tissue sampling or risk.

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References

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