Magnetic Resonance Imaging Demonstrates Incomplete Myelination in 18q- Syndrome: Evidence for Myelin Basic Protein Haploinsufficiency


1Department of Pediatrics, University of Texas Health Science Center at San Antonio, San Antonio, Texas
2Research Imaging Center, University of Texas Health Science Center at San Antonio, San Antonio, Texas
3Department of Radiology, University of Texas Health Science Center at San Antonio, San Antonio, Texas
4Department of Cellular and Structural Biology, University of Texas Health Science Center at San Antonio, San Antonio, Texas
5Department of Medicine, University of Texas Health Science Center at San Antonio, San Antonio, Texas

Magnetic resonance imaging (MRI) and MRI relaxometry were used to investigate disturbed brain myelination in 18q- syndrome, a disorder characterized by mental retardation, dysmorphic features, and growth failure. T1-weighted and dual spin-echo T2-weighted MR images were obtained, and T1 and T2 parametric image maps were created for 20 patients and 12 controls. MRI demonstrated abnormal brain white matter in all patients. White matter T1 and T2 relaxation times were significantly prolonged in patients compared to controls at all ages studied, suggesting incomplete myelination. Chromosome analysis using fluorescence in situ hybridization techniques showed that all patients with abnormal MRI scans and prolonged white matter T1 and T2 relaxation times were missing one copy of the myelin basic protein (MBP) gene. The one patient with normal-appearing white matter and normal white matter T1 and T2 relaxation times possessed two copies of the MBP gene. MRI and molecular genetic data suggest that incomplete cerebral myelination in 18q- is associated with haploinsufficiency of the gene for MBP. Am. J. Med. Genet. 74:422–431, 1997. © 1997 Wiley-Liss, Inc.

KEY WORDS: 18q- syndrome; white matter; delayed myelination; myelin basic protein

INTRODUCTION

18q- syndrome is a rare disorder characterized by mental retardation, dysmorphic features, growth failure, and abnormal cerebral white matter [Wertelecki and Gerald, 1971; Miller et al., 1990; Vogel et al., 1990; Gay et al., 1994]. Table I summarizes the most commonly reported clinical characteristics associated with this disorder. Neurologic manifestations include seizures, hypotonia, nystagmus, and poor coordination [Wertelecki and Gerald, 1971; Wilson et al., 1979; Felding et al., 1987]. Reported structural brain abnormalities include impaired migration with associated heterotopic gray and white matter, ventricular enlargement, and cerebellar hypoplasia [Vogel et al., 1990; Felding et al., 1987]. Neuroimaging [Miller et al., 1990; Weiss et al., 1991; Rodichok and Miller, 1992; Ono et al., 1994; Lebron et al., 1994] and neuropathologic [Felding et al., 1987; Vogel et al., 1990] studies demonstrate white matter abnormalities, typified by "delayed" myelination, in several patients. Abnormal myelination in 18q- is most likely the result of the absence of one or more genes that participate in myelogenesis. Normal cerebral myelination requires the coordinated expression of a number of genes, including the gene for myelin basic protein (MBP) [De Vellis, 1990]. The gene for MBP is near the telomere of chromosome 18q, the region most commonly deleted in 18q- syndrome [Kamholz et al., 1987]. Most patients with 18q- are deficient for one copy of this gene [DuPont et al., 1995]. Thus, we sought to investigate the possibility that abnormal brain myelination in people with 18q- syndrome correlates with MBP haploinsufficiency [Weiss et al., 1991; Miller et al., 1990; Ono et al., 1994; Wilkie, 1994]. Myelination begins in utero and continues into young adulthood, but most brain myelination occurs during infancy [Yakovlev and Lecours, 1967; Brody et al., 1987; Kinney et al., 1988]. Conventional spin-echo MRI readily demonstrates this developmental process.
In the patients, chromosome 18q deletions were variable, ranging from a proximal breakpoint at 18q21.1 to a more distal breakpoint at 18q23. Two patients, 8 and 13, had cytogenetically observable interstitial deletions. Preliminary data regarding physical [Ghidoni et al., 1994], endocrinological [Hale et al., 1995; Ghidoni et al., 1997], neuropsychological [Thompson et al., 1995], and neuroimaging [Gay et al., 1994] characteristics of this group have been reported previously.

This study was approved by the Institutional Review Board of the University of Texas Health Science Center at San Antonio.

**Neuroimaging Techniques**

**MRI acquisition.** All MR examinations of the brain were performed on a 1.9-T magnet (Elscint, Haifa, Israel) using conventional spin-echo techniques. Axial T1-weighted (500/20: TR/TE) and double-echo axial T2-weighted (3,400/20–80) images with identical slice alignment were obtained using 5-mm thick slices with a 1-mm gap and a 256 X 256 matrix. The T2-weighted studies used a second-order motion compensation pulse sequence during the second echo to compensate for pulsatile flow-induced artifacts. Patients who were unable to lie still were sedated with chloral hydrate, 50-100 mg/kg of body weight (maximum dose, 1,000 mg), and physiologic indices were monitored by a pulse oximeter.

**Qualitative MRI analysis.** Two investigators independently evaluated the MRI scans for degrees of cerebral myelination. The investigators were blinded to the patients' MBP genotype. The images were assigned a numerical score based on the general appearance of cerebral white matter, with higher numbers indicating more immature white matter. Patients' T2-weighted MR images were compared to age-matched normal images after scoring was completed. The following grading scale was used: grade 1, normal-appearing white matter (adult pattern); grade 2, white matter signal intensity higher than normal, but lower signal intensity than adjacent gray matter; grade 3, white matter isointense compared with gray matter; grade 4, white matter hyperintense compared with gray matter.

**Quantitative MRI analysis: MRI relaxometry.** Digitized raw image data were converted to quantitative T1 and T2 parametric image maps on a Sun Sparcstation (Sun Microsystems, Mountain View, CA) using Magnetic Resonance Parametric Analyzer (MRPA) software (Research Imaging Center, University of Texas Health Science Center at San Antonio) [Downs et al., 1992; Herndon, 1995]. This method for estimating T1 and T2 maps uses T1-weighted and dual-echo T2-weighted conventional spin-echo sequences commonly used clinically for MR imaging of the brain. MRPA calculates the parametric image maps using a look-up table. The look-up table contains pre-solved solutions to Bloch's equations for ratios of MR signals (images) across predefined ranges of T1 and T2 in milliseconds. The outputs of these calculations are estimated T1 and T2 parametric image maps with pixel

**MATERIALS AND METHODS**

**Human Subjects**

Twenty patients (11 female and 9 male) and 12 control subjects (9 female and 3 male) comprised a pilot group from a large multidisciplinary project investigating the molecular and clinical characteristics of patients with 18q- syndrome. Affected individuals were between 5 mo and 13.9 yr of age (mean age, 6.2 yr; SD 3.3 yr). Control subjects were normal children—as determined by history—between 3 mo and 13.3 yr (mean age, 7.0 yr; SD 4.1 yr). They were recruited locally or were siblings of patients and were scanned for this study, not for any clinical indication.

**TABLE I. Commonly Reported Features of 18q- Syndrome (From The 18q-Registry and Research Society, San Antonio, Texas)**

<table>
<thead>
<tr>
<th>Feature</th>
<th>%</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mental retardation</td>
<td>96</td>
<td>33</td>
</tr>
<tr>
<td>Short stature</td>
<td>80</td>
<td>32</td>
</tr>
<tr>
<td>Midface hypoplasia</td>
<td>70</td>
<td>30</td>
</tr>
<tr>
<td>Hypotonia</td>
<td>63</td>
<td>9</td>
</tr>
<tr>
<td>Prominent antihelix</td>
<td>63</td>
<td>17</td>
</tr>
<tr>
<td>Microcephaly</td>
<td>61</td>
<td>4</td>
</tr>
<tr>
<td>Carp mouth</td>
<td>57</td>
<td>28</td>
</tr>
<tr>
<td>Abnormal male genitalia</td>
<td>55</td>
<td>28</td>
</tr>
<tr>
<td>Foot deformity</td>
<td>48</td>
<td>26</td>
</tr>
<tr>
<td>Increased whorl patterns</td>
<td>44</td>
<td>26</td>
</tr>
<tr>
<td>Stenotic ear canals</td>
<td>41</td>
<td>26</td>
</tr>
<tr>
<td>Long tapering digits</td>
<td>39</td>
<td>26</td>
</tr>
<tr>
<td>Proximally set thumbs</td>
<td>35</td>
<td>26</td>
</tr>
<tr>
<td>Nystagmus</td>
<td>35</td>
<td>26</td>
</tr>
</tbody>
</table>

We used MRI and MRI relaxometry to characterize cerebral white matter development in 20 children with 18q- syndrome and in 12 normal children. We also determined the MBP genotype in the children with 18q- syndrome and in 12 normal children. We also determined the MBP genotype in the children with 18q- syndrome and in 12 normal children.

<ref>TABLE I. Commonly Reported Features of 18q- Syndrome (From The 18q-Registry and Research Society, San Antonio, Texas)</ref>
values in milliseconds (Fig. 1). Relaxometry results were calibrated by measuring T1 and T2 of standard copper sulfate solutions using MRPA and comparing T1 and T2 on the same copper sulfate concentrations determined with inversion recovery sequences.

T1 and T2 parametric image maps were transferred to a Macintosh (Apple Computer, Cupertino, CA) workstation for viewing and for region-of-interest (ROI) analysis using Digital Image Processing Station (DIPS) (HIPG, Boulder, CO). We performed analyses on several widely separated regions of the brain because the timing and rate of cerebral myelination vary regionally [Barkovich et al., 1988; Yakovlev and Lecours, 1967; Brody et al., 1987; Kinney et al., 1988]. ROIs were drawn in four white matter regions: minor forceps of the corona radiata (frontal white matter), major forceps of the corona radiata (occipital white matter), genu of the corpus callosum, and middle cerebellar peduncle. Homologous regions in the left and right hemispheres were combined to increase the size of each ROI, thereby reducing the impact of noise and of random variation. Combined homologous ROIs contained a minimum of 30 pixels. Mean T1 and T2 relaxation times are reported for four white matter regions.

Molecular Genetic Analysis

Fluorescence in situ hybridization (FISH). Metaphase chromosome preparations were obtained from each patient either from transformed lymphocytes or by primary blood harvest using the standard method of Moorehead et al. [1960] with ethidium bromide treatment to produce prometaphase spreads [Ikeuchi, 1984]. The MBP P1 probe was isolated by screening a human genomic P1 library [Shepherd et al., 1994] using a set of MBP-specific PCR primers, 4018 and 4017 (Primer sequence obtained from C. Chinault, Genome Database, Johns Hopkins University, 1992). The purified P1 DNA was labeled with biotin by nick translation using biotin-14-dATP (BRL/Gibco, Bethesda, MD). FISH was carried out as previously described [Pinkel et al., 1988] using 40 ng of P1 probe. The fluorescent probes were visualized using a Zeiss Axioplan Fluorescent microscope equipped with FITC, DAPI, and triple band pass filter sets. Images were digitized using Applied Imaging Probevision (Pittsburgh, PA), and photographs were printed on a Kodak XL 7700 color image printer.

Determination of deletion extent. Molecular analysis using genomic DNA was performed to deter-
mine the extent of deleted chromosome 18 material in all of the patients. Hybrid cell lines were constructed on key patients defining the critical region using previously described methods [Cody et al., 1997]. The DNA was then analyzed using polymerase chain reaction (PCR)-based markers from Genethon [Gyapay et al., 1994].

PCR was performed in a total reaction volume of 10 µl using 50 ng of genomic DNA, 50 ng of each primer, 200 mM dNTP's, 0.5 U Taq polymerase (Perkin Elmer-Cetus, Norwalk, CT), and 1.5 mM MgCl₂. One primer of the pair was end-labeled at the 5' end with γ³²P-dATP. PCR amplification consisted of 30 cycles of 1 min at 95°C, followed by 1 min at an annealing temperature of 60°C, and 1 min elongation at 72°C. PCR products were separated on a 7% polyacrylamide gel run at 65 W for 5 hr and visualized using Kodak XAR-5 film and intensifying screens.

**Statistical Analysis**

Statistical analyses were performed on MRI relaxometry data with SPSS [1993]. We analyzed the effect of age on white matter development using linear regression analyses of log-transformed data (all log-transformed data were normally distributed, Kolmogorov-Smirnov test, 2p ≥ 0.058). Log-transformation for all dependent and independent variables provided the best fit. Intercross of the regression lines for patients and controls were compared with the t-test. Slopes of the regression lines (regression coefficients) were tested with analysis of variance (ANOVA). When there are no significant differences between slopes (i.e., when slopes are parallel), differences between the intercepts of the regression lines of patients and controls measure the differences in T1 and T2 relaxation times between groups. Slopes of the regression lines assess age-related effects on T1 and T2.

The Mann-Whitney U test was used to compare overall differences in age between patients and controls.

**Combining homologous regions of interest.** We sought to increase ROI sample size and thus increase the accuracy of the estimate of the mean pixel value by combining samples from homologous left and right cerebral hemisphere regions. Using the paired-sample t-test, there were no differences in T1 (or T2) between homologous left and right hemisphere regions (P > 0.05). Thus, we report mean T1 (and mean T2) as single values for each of these paired regions.

**Interrater reliability for region of interest analyses.** Interclass correlation coefficients were used to evaluate interrater reliability for the region of interest measurements [Fleiss, 1986]. Three investigators independently determined T1 and T2 in the frontal and occipital white matter of five patients. Variance components for investigators, patients, and error were computed from an analysis of variance table. No significant mean differences were observed among raters (P > 0.05). Interclass correlations ranged from r = .898 for T2 measurements in the occipital white matter to r = .996 for associated T1 measurements. Three of the four interclass coefficients were r = .99 or higher. All four coefficients suggest excellent interrater reliability for these region of interest analyses.

**Interrater reliability for qualitative MRI analyses.** The simple kappa statistic was calculated to determine interrater reliability for qualitative analysis of patients' MRI scans [Fleiss, 1981]. A simple kappa score of 0.7 indicated that there was good agreement between the two investigators using the qualitative scale described above.

**RESULTS**

Age did not differ significantly between the patient group and the control group (Mann-Whitney U test, P = 0.55). MRI data from patient 8 was treated separately because this patient's genotype differed from the genotypes of all other patients regarding MBP gene copy number. The MBP gene copy number for all 20 patients was determined using FISH. Nineteen patients had only one copy of the gene for MBP (Fig. 2a). Patient 8 had two copies of the MBP gene (Figure 2b). Figure 3 demonstrates the deletion extent in three patients. Patients 8 and 13 had interstitial deletions of 18q. All other patients had terminal deletions, and patient 33 had the smallest deletion. In patient 8, a unique region of approximately 2 megabases was conserved that contained the gene for MBP. This region was missing from one copy of 18q in all other patients.

Qualitative MRI assessment was made by two independent raters. Investigators concurred on the degree of myelination for 16 patient scans; for each of the other four scans, all rated as abnormal by both investigators, an average grade was calculated. Nineteen of 20 patients' scans exhibited abnormal white matter compared to age-matched controls. The principle abnormality was a reduced gray-white distinction due to increased white matter signal intensity in patients. The white matter in the 5-month-old patient and in the 3-month-old control had similar signal intensities, but this patient's total cerebral white matter volume and corpus callosum size were reduced and posterior horn ventricular volume was increased (colpocephaly, Fig. 4a). For the 18q- group, gray-white contrast increased such that the scans appeared more mature-with increasing patient age (Fig. 4). However, gray-white contrast was poor and white matter T2-weighted signal intensity was abnormally high even in the oldest patient studied. The genu of the corpus callosum appeared normal in all but three patients. These findings contrasted with quantitative MRI results (see MRI relaxometry, below). Patient 8-the only patient with two copies of the gene for MBP-had a normal MRI scan (Fig. 2d). Brain MRI was normal in all 12 control subjects.

Quantitative evaluation of the MRI data using relaxometry revealed that white matter T1 and T2 relaxation times were significantly longer in patients than in controls. Age-related changes occurred in all regions (Fig. 5). Comparison of the intercepts for the log-transformed data demonstrated that T1 and T2 relaxation times were significantly longer in patients than in controls (t-test, P ≤ 0.001), while slopes of the regression lines for patients and controls were equal (ANOVA, p = 0.08). Thus, T1 and T2 remained prolonged even in older children with 18q-. Although the...
tween T1 (or T2) and age that were best described by exponential decay functions, such that 1) age effects on T1 and T2 were more pronounced at younger ages, and 2) T1 and T2 reached plateau values in early childhood (Fig. 5). Linear regression analysis of the log-transformed data revealed that the effect of age on T1 and T2 measurements was significant in all four regions studied in patients and in controls.

genu of the corpus callosum appeared normal on standard MR images in all but three patients, regression analysis demonstrated significant differences in corpus callosum T1 and T2 values between patients and controls (t-test, P < 0.001).

T1 and T2 relaxation times decreased with increasing age in both patients and controls. For both groups, scattergrams demonstrated nonlinear relationships between T1 (or T2) and age that were best described by exponential decay functions, such that 1) age effects on T1 and T2 were more pronounced at younger ages, and 2) T1 and T2 reached plateau values in early childhood (Fig. 5). Linear regression analysis of the log-transformed data revealed that the effect of age on T1 and T2 measurements was significant in all four regions studied in patients and in controls.

**Fig. 2.** FISH and MRI results for two 8-year-olds with 18q-. Both children had many characteristics of 18q- syndrome, including dysmorphic features and mental retardation. However, patient 18 had only one copy of the gene for MBP (a), while patient 8 had two copies of this gene (b). T2-weighted MRI images demonstrate abnormally increased signal intensity of the deep cerebral white matter in patient 18 (c), while the MRI of patient 8 is normal (d). Arrows in (a) and (b) point to FISH probe hybridizing to the MBP gene.
Patient 8 was the only 18q- patient with two copies of the gene for MBP. T1 and T2 relaxation times were in the normal range in all four white matter ROIs in this individual. Plots of T1 and T2 vs. age demonstrated that mean T1 and T2 values in all regions were age-appropriate (Fig. 5).

**DISCUSSION**

Conventional spin-echo MRI scans and quantitative MR relaxometry techniques demonstrated abnormal cerebral and cerebellar white matter—best described as incomplete myelination—in all of our patients with one copy of the MBP gene. MBP is crucial for normal myelogenesis, a major developmental process occurring primarily in the first 18 months of life [De Vellis, 1990; Wrabetz et al., 1990; Kamholz et al., 1988]. Several case reports suggested an association between abnormal myelination and MBP hemizygosity in patients with 18q- [Miller et al., 1990; Weiss et al., 1991; Vogel et al., 1990; Ono et al., 1994]. Miller et al. [1990] postulated that, in patients with 18q- syndrome, “impaired myelination of the central white matter tract...is most likely due to failure of expression of the MBP gene.” Our findings agree with this assertion. Furthermore, our only patient who had normal-appearing white matter and normal white matter T1 and T2 indices also had two copies of the MBP gene. In contrast to Miller et al. [1990], we found evidence for age-related maturation in white matter, as well as evidence for incomplete myelination in the corpus callosum. This finding is most likely because our analysis included younger patients than did Miller’s group, since we demonstrated the most pronounced age-related changes between five months and four years of age.

Reduced MBP gene dosage separated the MRI scans of 18q- patients from the scan of patient 8 and from the scans of normal controls. All patients with only one copy of the MBP gene had abnormal white matter indices. Furthermore, T1 and T2 relaxation curves plateaued at significantly higher levels in these patients than in controls, suggesting that white matter T1 and T2 indices in MBP-deficient patients do not normalize. Many authors refer to this type of white matter abnormality as “delayed myelination” [Ono et al., 1994; Miller et al., 1990; Barkovich, 1990; Bird et al., 1989]. Delayed myelination implies that myelination begins later in affected patients but eventually becomes normal or complete. Our MRI relaxometry results demonstrate that the phrase “incomplete myelination” is a more accurate descriptor for the white matter abnormality in this disorder. That is, T1 and T2 did not approach normal values, suggesting that the total myelin content in brain white matter was reduced at all ages in 18q- patients, a finding in agreement with previous neuropathological studies [Felding et al., 1987; Vogel et al., 1990].

Although white matter signal intensity was higher than normal at all ages, T2-weighted MRI scans demonstrated age-related white matter maturational changes in 18q- patients. Similarly, white matter T1 and T2 values decreased steadily until about four years of age, but these values did not approach control T1 and T2 values. The trend for decreasing T1 and T2 with increasing age in 18q- patients suggests that some events contributing to myelinogenesis are occurring. These events probably include expression of myelin components regulated by genes at loci not disrupted by 18q deletions [De Vellis, 1990].

Haploinsufficiency, resulting from reduced gene dosage, expression, or protein activity, is a common mechanism of disease in chromosomal deletions [Wilkie, 1994]. When a copy of the MBP gene is lost, a dominant phenotype occurs, consistent with a loss-of-function mutation. Therefore, the MBP gene appears to provide another example of a haploinsufficient locus. Reduced copy number of the MBP gene may lead to decreased production of MBP, a protein produced in large quantities by oligodendrocytes during infancy and early childhood. Since MBP is an essential component of normal myelin, decreased MBP availability might cause either reduced quantities of myelin, functionally abnormal myelin, or both.

A specific deleted critical region—not deletion size—leads to abnormal myelination in this disorder. Patient 8 had a large interstitial deletion sparing the gene for MBP. Despite the size of the deletion, this patient’s white matter appearance and T1 and T2 indices were
Fig. 4. Age-matched T2-weighted MRI scans, patients with the 18q- syndrome (a,c,e,g) and controls (b,d,f,h). A 5 month old patient (a) is compared to a 3 month old control (b). Scans c and d are from an 18 month old patient and control; e and f, 5 year olds; g and h, 10 year olds. Scans b,d,f and h depict the normal changes in gray-white contrast as myelination proceeds, with white matter achieving an essentially adult appearance by about 18 months of age. In patients with 18q- having only one copy of the gene for MBP, white matter signal intensity decreases with increasing age, but never normalizes, suggesting incomplete myelination. (Qualitative MRI scores for patients are: a = 4; c = 4; e = 3; g = 2. Scores for controls are: b = 4 [normal infant pattern]; d, f, and h = 1.)
Fig. 4. (Continued).
normal even though the deletion included chromosomal material also deleted in other 18q− patients. Molecular analysis revealed that the deleted chromosome 18q in patient 8 conserved a unique region of approximately 2 Mb not found in any of our other patients—the critical region for normal myelination in 18q−.

Kline et al. [1993] suggested that other genes on 18q nonadjacent to the gene for MBP may influence the degree of abnormal myelination. They reported normal MRI scans in two of five patients with distal 18q deletions that appeared to include the region encoding the MBP gene. However, they did not use quantitative MRI techniques, nor did they perform a specific assay for the MBP gene. Using visual MRI inspection alone, they could have missed more subtle white matter abnormalities in these two patients.

In addition to better characterizing incomplete myelination, MRI relaxometry detects white matter abnormalities that might not be detected visually using conventional MRI. Several authors reported that the corpus callosum appeared normal in 18q− patients even if adjacent central white matter exhibited abnormally increased signal intensity using T2-weighted images [Miller et al., 1990; Kline et al., 1993; Ono et al., 1994]. We demonstrated that T1 and T2 relaxation times in the genu of the corpus callosum were significantly longer in our patients than in age-matched controls even when this structure appeared normal.

Thus, MRI and MRI relaxometry data support the hypothesis that loss of a copy of the MBP gene is strongly associated with incomplete myelination in 18q− syndrome. Ongoing MRI studies include analyzing a group of very young patients and controls to characterize the nature of white matter maturation in 18q− in the first 12 months of life; performing serial follow-up MRI on 18q− patients to analyze longitudinally the effects of age on myelination patterns; and further characterizing white matter abnormalities in 18q− using magnetization transfer [Doussset et al., 1992; Hieble et al., 1994] and diffusion [Nomura et al., 1994; LeBihan et al., 1991] techniques. Continuing molecular characterization of the terminal region of 18q will help determine if other tightly linked deleted genes might contribute to incomplete myelination in this disorder.

ACKNOWLEDGMENTS

We thank Dr. Charles Dreyer for assistance in validating our data analysis methods and Dr. Daniel Hale for critically reviewing the manuscript. We also thank Ivelisse Galarza-Torres and Dr. Jinqui Li for assistance in acquiring the MRI images, and Cono Farias and Shawn Mikiten for assistance with producing the figures. This work was supported by funding from the Morrison Trust and the MacDonald family with matching funds from Microsoft, Inc.

REFERENCES


SPSS (1993): "SPSS." Chicago: SPSS.


