

# Proton Spectroscopy in Myotonic Dystrophy

## Correlations With CTG Repeats

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**Objectives:** To seek cerebral metabolite abnormalities in patients with myotonic dystrophy and to determine whether the degree of cerebral abnormalities (measured by proton magnetic resonance spectroscopy) correlates with severity of the genetic defect (measured by trinucleotide repeats).

**Design:** Fourteen patients with myotonic dystrophy were compared with 24 healthy control subjects.

**Setting:** A university-affiliated medical center.

**Results:** Compared with healthy subjects, patients with myotonic dystrophy had elevated levels of myoinositol (+19% in the occipital region and +12.9% in the temporoparietal region), total creatine (+7.6% and +6.8%), and choline-containing compounds (+21% and +7.7%). Furthermore, the creatine and myoinositol peak areas cor-

related with the number of trinucleotide cytosine-thymine-guanine (CTG)<sub>n</sub> repeats from leukocytes, especially in the temporoparietal brain region ( $r=0.76$ ;  $P=.004$ ).

**Conclusions:** Neurochemical alterations observed with proton magnetic resonance spectroscopy are proportional to the cytosine-thymine-guanine repeat size. Increases in myoinositol and creatine concentrations may be caused by increased glial content, while elevated levels of choline-containing compounds are most likely caused by increased glial content and cell membrane abnormalities. Proton magnetic resonance spectroscopy is a powerful noninvasive tool to study brain biochemistry, which may reflect the extent of neuropathological involvement in patients with myotonic dystrophy.

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**M**YOTONIC dystrophy is a progressive multisystem genetic disorder with an abnormality on the long arm of chromosome 19.<sup>1,2</sup> The genetic defect consists of an excessive trinucleotide repeat—cytosine-thymine-guanine (CTG)<sub>n</sub>, which renders the defective production of myotonin, an inferred substrate of the myotonic dystrophy protein kinase.<sup>3-5</sup> A gain of function by the myotonic dystrophy protein kinase RNA and effects of the CTG expansion on a candidate flanking gene, myotonic dystrophy locus-associated homeodomain protein, may also contribute to the pathophysiology of myotonic dystrophy.<sup>6,7</sup> The disease is characterized by myotonia, distal muscular weakness, and a variety of systemic disorders including cataracts, cardiac conduction defects, sleep apnea, endocrine abnormalities, and cognitive impairment.<sup>8</sup> However, the clinical expression of the disease and the age at onset of the illness are highly variable. Between 25% and 70% of these patients are

intellectually impaired,<sup>9,10</sup> but the cause of the cognitive deficits is unexplained.

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A variety of cerebral abnormalities, including cognitive impairment, abnormal neuroimaging findings on computed tomography, magnetic resonance (MR) imaging, single photon emission computed tomography, positron emission tomography, and neuropathological findings, have been reported in patients with myotonic dystrophy. Neuropsychological tests have shown lowered global intelligence (IQ) and personality changes<sup>11-14</sup> and, in particular, difficulties with visuospatial function and "frontal lobe" tasks.<sup>15-17</sup> The more severe cases demonstrated alterations of language and memory.<sup>14,18</sup> Neuroimaging studies have shown abnormalities such as a higher prevalence of periventricular hyperintensities on MR imaging,<sup>13,19-21</sup> cerebral hypoperfusion on single photon

## SUBJECTS AND METHODS

### SUBJECT SELECTION AND EVALUATION

Fourteen patients with a clinical diagnosis of myotonic dystrophy (8 men and 6 women, aged  $37.8 \pm 2.7$  years; **Table 1**) and 24 healthy control subjects (14 men and 10 women, aged  $39.6 \pm 2.4$  years) were consecutively recruited and studied with MR imaging and localized  $^1\text{H}$  MRS. Each patient underwent a screening evaluation that included medical and family histories; physical and neurologic examination (including a Folstein Mini-Mental State Examination<sup>27</sup>); electromyography; and an extensive laboratory battery, including complete blood cell count, routine chemistry studies, measurement of serum creatine (CR) and creatinine, thyroid panel, and measurement of fasting glucose and insulin, estrogen or testosterone, and serum and urine cortisol. Each patient with myotonic dystrophy also had DNA analysis for the CTG trinucleotide repeats by methods previously reported with polymerase chain reaction amplification and Southern blot analysis of restriction endonuclease digested genomic DNA.<sup>28,29</sup> The myotonic dystrophy analysis, as performed in this study, is greater than 99% accurate and identifies greater than 99% of all myotonic dystrophy mutations. Before the study, each subject and his or her guardian received a verbal description of the study procedures and signed an informed consent approved by the Human Subjects Institutional Review Board at Harbor-UCLA Research and Education Institute, Torrance, Calif.

### MR IMAGING AND LOCALIZED $^1\text{H}$ MRS

Each patient and subject underwent MR imaging to assess possible structural brain abnormalities. Both MR imaging and  $^1\text{H}$  MRS were performed on a clinical 1.5-T scanner (Signa 5.4, General Electric Co, Milwaukee, Wis) with the use of a quadrature head resonator. The examination began with the acquisition of a sagittal  $T_1$ -weighted localizer (echo time [TE], 11 milliseconds; repetition time [TR], 500 milliseconds; 4-mm slice thickness; 1-mm gap; 24-cm field of view), followed by a coronal fast double spin-echo sequence (TE1, 17 milliseconds; TE2, 102 milliseconds; TR, 4000 milliseconds; 5-mm slice thickness; no gap; 24-cm field of view). Finally, an axial fast inversion recovery scan (TE, 32 milliseconds; inversion time, 120 milliseconds; TR, 4000 milliseconds; 3.5-mm slice thickness; no gap; 24-cm field of view) was performed.

The  $^1\text{H}$  MRS was performed in 2 cortical regions: the midoccipital gray matter and the left temporoparietal gray

matter regions (**Figure 1**). Voxel sizes ranged between 3 and 5  $\text{cm}^3$ , depending on the individual anatomy of the subject; voxel sizes and location were carefully chosen by one of us (L.C.) to ensure consistent placement and to ascertain that each voxel contained primarily gray matter. After shimming and optimizing the water suppression, data were acquired by means of a double spin-echo sequence, point resolved spectroscopy (PRESS),<sup>30</sup> with TE of 30 milliseconds, TR of 3 seconds, 128 averages, 2048 time points, and 2.5-kHz band width. The PRESS sequence was optimized for the chosen echo times and locations.<sup>31</sup> To avoid the ambiguities caused by the use of metabolite ratios, metabolite concentrations were determined.<sup>32,33</sup> Briefly, the  $T_2$  decay of the unsuppressed water signal from the PRESS experiment was measured at 10 different echo times (from 30 milliseconds to 1.5 seconds; TR, 20 seconds). The signal from an external standard consisting of pure water also was acquired. This made it possible to determine the compartmentation within the selected volume (partial volumes of cerebrospinal fluid, visible brain water, and an MR-invisible compartment) and to calculate absolute metabolite concentrations corrected for the partial volume of cerebrospinal fluid. This approach yielded interindividual variations of about 10% for the major peaks. Furthermore, this procedure showed an intrasubject variability of 3% for measurements of CR and 8% for the measurements of choline-containing compounds (CHO).

The data were transferred to a SPARC 2 workstation and processed by means of the GE spectroscopy analysis platform (General Electric Medical Systems, Milwaukee and a semiautomatic program.<sup>32,33</sup> All data were processed by one of us (T.E.), who was blinded to the diagnosis of the subjects. These measurements yielded metabolite concentrations in "institutional units," which were converted into millimoles per kilogram concentrations by means of the published normal values in occipital cortex.<sup>33</sup> To compare our data with those previously reported, we also determined metabolite ratios with CR used as an internal standard.

Statistical analyses were performed with Statview (Abacus Inc, Berkeley, Calif). Descriptive analyses were performed on each metabolite. Comparisons between patients with myotonic dystrophy (excluding patient 3) and healthy subjects for each brain metabolite in each brain region were performed by Student *t* tests. Patient 3 was excluded in the comparison test because of a lack of an appropriate age-matched control. Regression analyses of metabolite concentrations on the trinucleotide repeats, and between different metabolites, were performed by means of a linear regression model. All values in the text and tables are reported as mean  $\pm$  SE; *P* values less than .05 are considered significant, while *P* values between .05 and .10 are considered a trend for significance.

emission computed tomography,<sup>18</sup> and cerebral hypometabolism on positron emission tomography<sup>22</sup> in these patients. Neuropathological examination of brains of patients with myotonic dystrophy found neurofibrillary tangles,<sup>23,24</sup> with abnormally phosphorylated  $\tau$  protein<sup>25</sup> and intracytoplasmic inclusions.<sup>26</sup> Taken together, these findings clearly demonstrate that brain abnormalities occur in addition to the neuromuscular involvement in myotonic dystrophy.

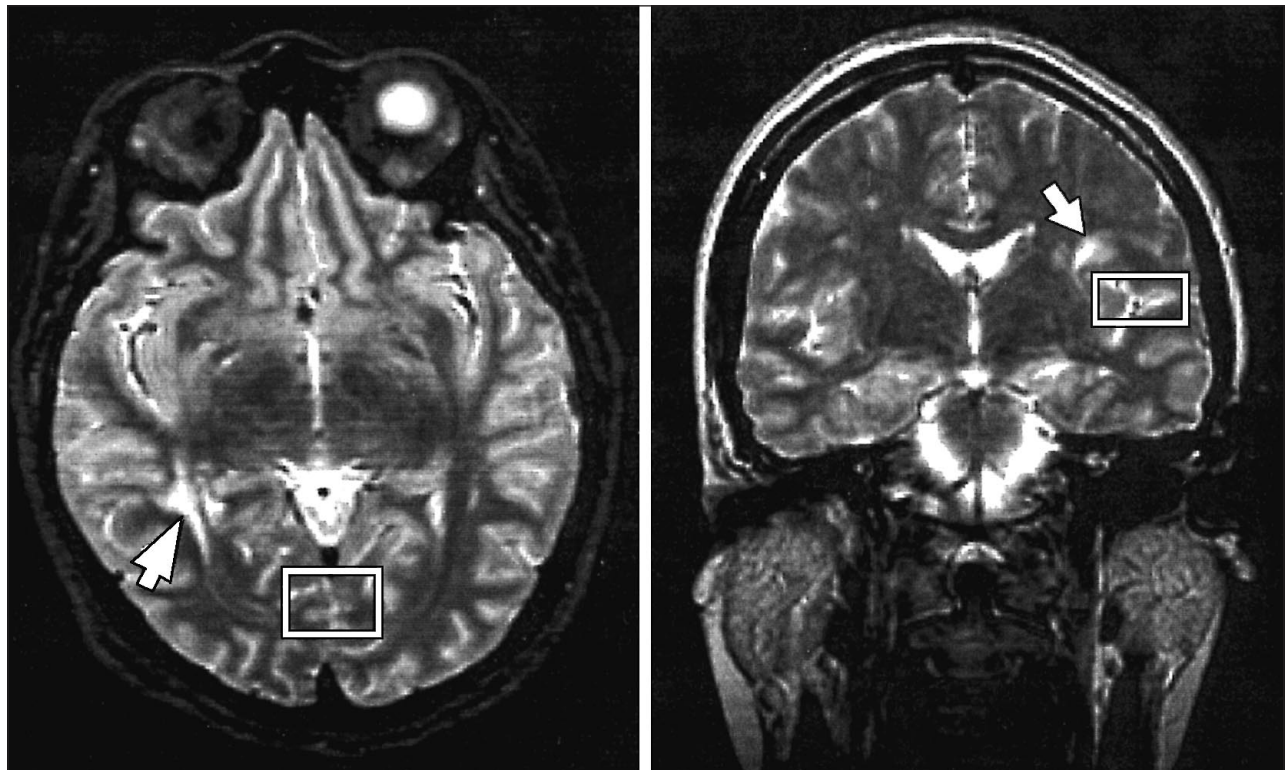
Magnetic resonance spectroscopy (MRS) can measure in vivo cerebral biochemistry and may help to further elucidate the cerebral pathophysiological characteristics of the disease. This study aimed to evaluate possible cerebral metabolite abnormalities in patients with myotonic dystrophy by means of proton ( $^1\text{H}$ ) MRS. We also determined whether the genetic abnormalities, as measured by trinucleotide repeats, correlated with metabolite levels in the brain.

**Table 1. Age, Sex, and Clinical and MR Imaging Characteristics of Patients With Myotonic Dystrophy\***

Family	Patient No./ Sex/Age, y	Inheritance Pattern	No. of CTG Repeats	Age at Onset, y	Duration of Illness, y	MMSE Score†	MR Imaging Findings
I	1/F/34	Paternal	1140	32	2	18	Minimal PVWML at posterior horns
I	2/F/29	Paternal	1081	26	3	19	Normal
I	3/F/2.5	Maternal	1434	0	2.5	...	Mild generalized atrophy
I	4/M/33	Paternal	788	20	13	21	Minimal PVWML at posterior horns
I	5/M/39	Paternal	893	15	24	28	Mild PVWML at anterior horns
I	6/F/36	Paternal	802	18	18	29	Normal
II	7/M/42	Paternal	826	14	28	30	Normal
I	8/M/37	Paternal	1061	35	2	26	Mild PVWML at posterior horns
I	9/F/31	Paternal	816	25	6	30	Normal
III	10/M/48	Maternal	690	38	10	28	Mild bilateral parietal atrophy; mild PVWML at posterior horns
IV	11/M/62	Paternal	861	40	22	28	Mild parietal atrophy; moderate PVWML at posterior horns
IV	12/M/32	Paternal	444	27	5	27	Normal
V	13/F/30	Paternal	173	25	5	28	Moderate bilateral subcortical WML; moderate PVWML at posterior horns
VI	14/M/50	Paternal	...	31	19	27	Normal

\*MR indicates magnetic resonance; MMSE, Mini-Mental State Examination; WML, white matter lesions; PVWML, periventricular white matter lesions; and ellipses, not available.

†Maximum score, 30.



**Figure 1.** Left, Axial inversion-recovery magnetic resonance image showing the typical location of the midoccipital gray matter voxel; note a discrete hyperintense white matter lesion adjacent to the right posterior horn of the lateral ventricle (white arrow). Right, Coronal T<sub>2</sub>-weighted magnetic resonance image showing the left temporoparietal gray matter voxel; note also a discrete hyperintense white matter lesion in the subcortical parietal brain region (white arrow).

## RESULTS

### PATIENT CHARACTERISTICS

The patients with myotonic dystrophy had a duration of illness of  $13.8 \pm 3.5$  years, with a mean age at onset of the illness of  $24.1 \pm 3.2$  years. Twelve of 14 patients had paternal inheritance of the disease. Neither age at onset nor

duration of illness correlated with CTG repeats. However, Mini-Mental State Examination scores showed a trend for correlation with CTG repeats ( $r=0.48$ ;  $P=.10$ ). In addition, serum creatinine level correlated with age at onset of illness ( $r=0.69$ ;  $P=.006$ ) but not with duration of illness or any of the brain metabolites. Table 1 shows some clinical findings of the patients with myotonic dystrophy. Seven patients had cataracts and, ex-

**Table 2. Metabolite Concentrations From Proton MRS in Patients With Myotonic Dystrophy and Healthy Control Subjects\***

	Myotonic Dystrophy (n=13)	Healthy Control Subjects (n=24)	P†
<b>Midoccipital Gray Matter</b>			
NA	9.29±0.33 (-2.0%)	9.48±0.20	.54
CR	9.25±0.30 (+7.6%)	8.60±0.18	(.08)
CHO	1.83±0.09 (+21.2%)	1.51±0.09	.01
MI	9.48±0.30 (+19.1%)	7.96±0.36	.005
NA/CR	1.35±0.04	1.61±0.03	<.0001
CHO/CR	0.58±0.02	0.53±0.01	(.06)
MI/CR	0.69±0.02	0.66±0.02	.19
GLX/CR	0.48±0.02	0.48±0.01	.96
% CSF	10.5±1.3 (+28%)	8.2±0.5	(.06)
<b>Temporoparietal Gray Matter</b>			
NA	9.29±0.13 (0%)	9.29±0.33	.98
CR	9.37±0.18 (+6.7%)	8.78±0.24	.05
CHO	2.22±0.06 (+7.8%)	2.06±0.06	(.08)
MI	9.61±0.24 (+12.9%)	8.51±0.30	.01
NA/CR	1.29±0.02	1.48±0.03	<.0001
CHO/CR	0.73±0.02	0.70±0.02	.20
MI/CR	0.70±0.02	0.67±0.01	(.1)
GLX/CR	0.50±0.01	0.50±0.02	.98
% CSF	12.2±1.7 (+27%)	9.6±0.8	.18

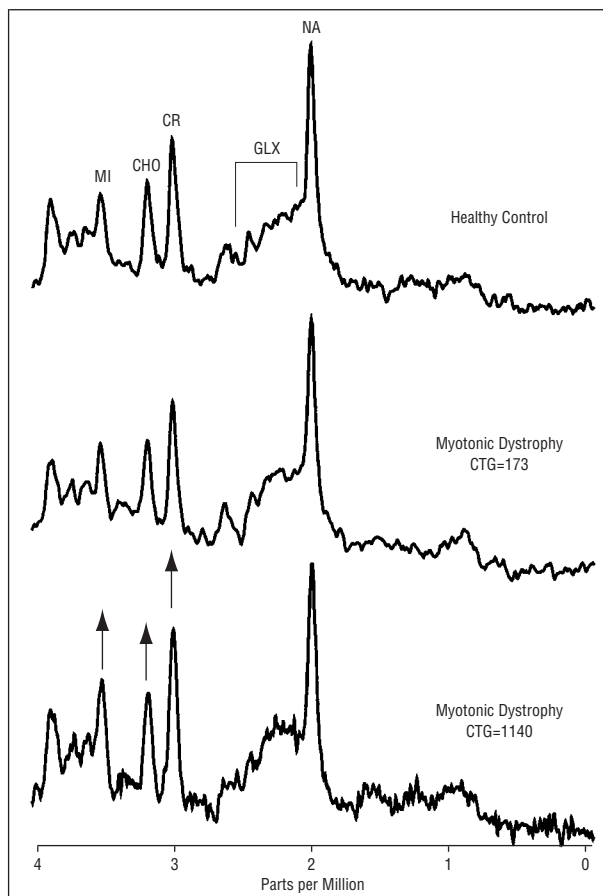
\*MRS indicates magnetic resonance spectroscopy; NA, N-acetyl compounds; CR, creatine; CHO, choline-containing compounds; MI, myoinositol; GLX, glutamate/glutamine; and % CSF, percentage of cerebrospinal fluid. Values are mean±SE (percentage of change in the metabolites in patients with myotonic dystrophy relative to the metabolites in healthy control subjects). Metabolite concentrations are in millimoles per kilogram.

†P values in parentheses indicate trends for significance.

cept for 1 patient (patient 11), all had percussion or grip myotonia in their hypothenar muscles. All patients had facial diparesis, variable severity of muscle weakness with atrophy in the distal extremities, and hyporeflexia. The electromyogram showed characteristic myotonia in all patients except for patient 3. All of the male patients showed frontal balding except for patient 14. Low estrogen level of 136 pmol/L (normal, 224-1285 pmol/L) was detected in patient 1. Three of the 7 male patients (patients 7, 10, and 12) showed reduced testosterone levels of 7.3 to 8.0 pmol/L (2.1-2.3 ng/mL) (normal, 10.4-34.7 pmol/L [3.0-10.0 ng/mL]). Overnight urine cortisol levels were found to be low in 2 patients (patients 5 and 8), while PM cortisol level was elevated in 2 other patients (patients 4 and 10). Insulin resistance was found in 4 subjects (patients 4, 7, 9, and 12), as reflected by elevated insulin levels of 189 to 458 pmol/L (normal, 29-158 pmol/L) with normal serum glucose levels. No thyroid hormone abnormalities were noted except in patient 7, who was receiving thyroid hormone replacement. Patient 2 gave birth to patient 3, who was a floppy infant at birth.

#### MR IMAGING AND <sup>1</sup>H MRS RESULTS

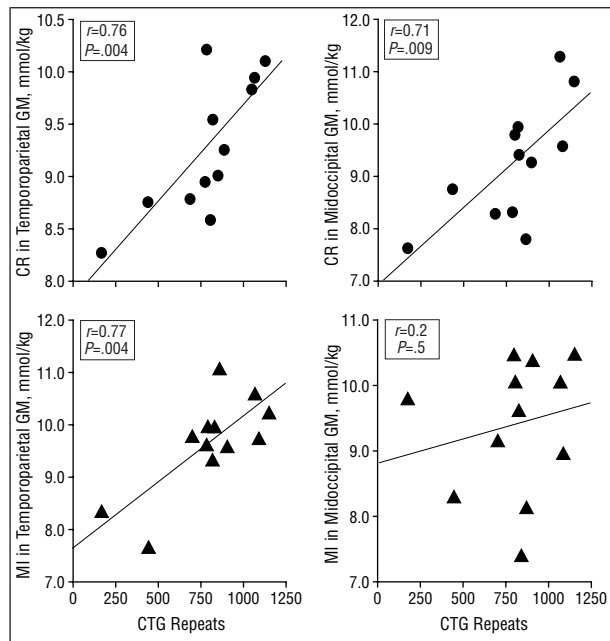
Magnetic resonance images of all the healthy subjects exhibited no abnormalities, while MR images of only 7 of the patients with myotonic dystrophy exhibited as completely normal. The other 7 patients showed minimal to mild amounts of confluent periventricular white matter



**Figure 2.** Proton magnetic resonance spectroscopy from the temporoparietal gray matter voxels of a healthy control subject (upper spectrum), a patient with myotonic dystrophy with 173 cytosine-thymine-guanine (CTG) repeats (middle spectrum), and a patient with myotonic dystrophy with 1140 CTG repeats (lower spectrum). Significantly increased levels of creatine (CR), choline-containing compounds (CHO), and myoinositol (MI) are observed in the subject with a higher number of CTG repeats. GLX indicates glutamate/glutamine; NA, N-acetyl compounds.

lesions (Table 1 and Figure 1). These lesions were located primarily adjacent to the posterior horn of the lateral ventricles and appeared minimally to mildly hyperintense on both the T<sub>2</sub>-weighted and the inversion-recovery MR images. No significant atrophy, infarcts, or other focal lesions were noted.

**Table 2** shows the cerebral metabolite concentrations in patients with myotonic dystrophy and in healthy subjects (**Figure 2**). N-acetyl compounds (NA) and glutamate/glutamine were normal in both the midoccipital and the temporoparietal cortical brain regions of the patients with myotonic dystrophy. However, NA showed a trend for a negative correlation with duration of illness ( $r=-0.45$ ;  $P=.1$ ). Myoinositol (MI) concentration was significantly increased in both cortical regions (19% and 12.9%). The MI level in the temporoparietal brain region also showed a trend for correlation with duration of illness ( $r=0.37$ ;  $P=.1$ ). In addition, CR and CHO levels were elevated in both brain regions, but no correlation with duration of illness was observed. To confirm the quantitative values, we also analyzed the data by means of metabolite ratios with CR as the internal reference. We found a lower NA/CR ratio in both brain regions; these lower values resulted from the relatively normal NA and elevated CR levels. The ratios of



**Figure 3.** Regression analysis between the metabolite concentrations and the trinucleotide (cytosine-thymine-guanine [CTG]) repeats. CR indicates creatine; GM, gray matter; and MI, myoinositol.

CHO/CR were relatively normal in both brain regions, since both CHO and CR levels were elevated. However, the MI/CR ratio was higher, although not significantly, in both brain regions, which agrees with the finding that the increase in MI level is greater than the increase in CR level. None of the metabolite ratios (NA/CR, CHO/CR, and MI/CR) in either brain region correlated with age, age at onset, or duration of illness, except for MI/CR in the temporoparietal brain region, which correlated with age at onset ( $r=0.57$ ;  $P=.03$ ).

In patient 3, the only patient with the congenital form of myotonic dystrophy, we found mildly elevated CHO and lower NA levels but no elevation of MI or CR level in either brain region when compared with the adult controls. Patients with hormonal abnormalities did not show significantly different metabolite abnormalities compared with those without hormonal alterations.

To further evaluate the relationship between the metabolite abnormalities and the genetic defects, we performed regression analyses of the metabolites on the CTG repeats. We found significant correlations between CTG repeats and CR in both the occipital cortex ( $r=0.71$ ;  $P=.009$ ) and the temporoparietal cortex ( $r=0.76$ ;  $P=.004$ ; **Figure 3**). We also found a significant correlation between CTG repeats and MI level in the temporoparietal cortex ( $r=0.77$ ;  $P=.004$ ) but not in the occipital cortex (Figure 3). Although CHO level was elevated in the patients with myotonic dystrophy, no significant relationship was found with the number of the CTG repeats. The NA level did not correlate with the number of CTG repeats. The Mini-Mental State Examination score showed a trend for correlation with CTG repeats but no correlation with any of the brain metabolites.

We also observed a relationship between MI and CR levels in the temporoparietal brain region ( $r=0.77$ ;  $P=.002$ ) and a trend in the occipital cortex ( $r=0.49$ ;  $P=.09$ ) of the

patients with myotonic dystrophy. The MI level did not correlate with CHO level in either brain region in these patients.

## COMMENT

This study found mild and nonspecific abnormalities on MR imaging and abnormal cerebral metabolite concentrations on  $^1\text{H}$  MRS in a group of patients with myotonic dystrophy. The MR imaging abnormalities are in agreement with previous reports of patients with myotonic dystrophy,<sup>34</sup> except that our patients showed primarily periventricular white matter hyperintensities adjacent to the posterior horns of the lateral ventricles (temporoparietal white matter). This finding is consistent with neuropathological and other neuroimaging reports that the temporoparietal or temporal lobe regions are the most severely affected in myotonic dystrophy.<sup>18,25</sup>

For the MRS study, the 2 voxel locations, temporoparietal gray matter and midoccipital gray matter, were chosen because previous single photon emission computed tomographic studies showed primarily temporoparietal hypoperfusion in patients with paternally inherited myotonic dystrophy,<sup>18</sup> and because neuropathology studies also documented temporal lobe and occipital cortex abnormalities.<sup>23-25</sup> In both the occipital and temporoparietal brain regions, we found increased levels of MI, CR, and CHO and normal NA levels. The NA/CR ratio, however, was significantly lower because of the elevated CR level. Our finding of a decreased NA/CR ratio is consistent with the only previous MRS study in myotonic dystrophy.<sup>35</sup> In that study, Hashimoto et al<sup>35</sup> examined 5 pediatric patients with congenital myotonic dystrophy and found decreased NA/CHO and NA/CR ratios in various brain regions (occipital, frontal, and parietal regions containing both gray and white matter in each voxel). They concluded that NA level was decreased in their patients, possibly as a result of a developmental disorder of neurons in myotonic dystrophy. There are 2 possible reasons for the different conclusions from their study: (1) The patients studied by Hashimoto et al had congenital myotonic dystrophy, while all of our patients (except for patient 3, who was excluded from the comparison studies) had adult-onset, and primarily paternally inherited, myotonic dystrophy. (2) Hashimoto et al used only metabolite ratios rather than the individual metabolite concentrations; it is also difficult to compare their study with ours since they used a different method than we did, long echo time measurements (TE, 270 milliseconds) vs our short echo time study (TE, 30 milliseconds). However, our measurements demonstrate no significant decreases in NA levels; the decreased NA/CR and NA/CHO ratios primarily result from increased CR and CHO levels. The relatively normal NA level reflects minimal neuronal loss or dysfunction in our patients. We observed a trend for NA to decrease with duration of illness, which indicates possible neuronal loss with the progression of the disease.

Furthermore, levels of CR and MI, but not NA or CHO, positively correlated with the number of trinucleotide repeats. There are several possible explanations for these correlations. First, because MI has been identified as a glial-specific marker,<sup>36</sup> elevated MI level suggests increased glial content. In addition, recent studies showed

that CTG repeats correlate with clinical disease severity on neurologic examination.<sup>37,38</sup> Consequently, the positive correlation of MI level with CTG repeats suggests that an increase in glial content is related to the severity of the disease. Elevated MI level has also been observed in numerous diseases, such as multiple sclerosis,<sup>39</sup> dementia associated with human immunodeficiency virus type 1,<sup>40,41</sup> progressive multifocal leukoencephalopathy,<sup>42</sup> Alzheimer disease,<sup>43</sup> and frontotemporal dementia.<sup>44</sup> All of these diseases show increased glial activity because of either glial hypertrophy associated with repair processes or gliosis in regions with neuronal loss. Furthermore, MI also is known to be an osmolyte<sup>45</sup>; its role in the brain may be for the maintenance of cell volume in reactive astrocytes.<sup>46</sup>

Glial proliferation associated with neuronal degeneration has been shown throughout the cerebral cortex of myotonic dystrophy.<sup>23</sup> In some brain regions, such as the hypothalamus and brainstem, marked gliosis was associated with well-preserved neurons.<sup>23</sup> Others observed intracytoplasmic inclusion bodies in the thalamus<sup>26,47</sup> as well as in the cortex, putamen, and caudate; these were thought to be specific for the pathogenesis of myotonic dystrophy, since they were found in much higher numbers than in the controls.<sup>47</sup> Recent studies also reported neurofibrillary changes similar to those of Alzheimer disease in the limbic and insular cortical regions in brains of patients with myotonic dystrophy.<sup>23</sup> The presence of abnormally phosphorylated  $\tau$  protein, most prominently in the temporal lobes and different from those found in Alzheimer disease, also has been reported.<sup>25</sup> Our findings of metabolite abnormalities in the temporoparietal region are consistent with neuropathological and immunohistochemical observations that the temporal lobes are affected in myotonic dystrophy. However, we find additionally that the midoccipital gray matter regions are abnormal.

The mechanisms underlying the increased CR level and the correlation of CR level with CTG repeats are less clear. There are 2 possibilities for the increased CR peak. First, there might be a relationship between the severity of the trinucleotide repeats and CR uptake into the brain, since CR is synthesized only in the liver. This increased uptake may in turn result from an abnormality in the CR transporter, which is in the same family of sodium- and chloride-dependent transporters for the uptake of choline.<sup>48</sup> In nondystrophic myotonic diseases, both chloride and sodium channel defects have been shown in muscle<sup>49-51</sup>; whether these abnormalities also occur in myotonic dystrophy is unknown. In parallel with elevated MI level, elevated CR level has been observed in the adjacent white matter of lesions in multiple sclerosis<sup>52</sup> or progressive multifocal leukoencephalopathy<sup>42</sup> and in the white matter of cocaine users.<sup>33</sup> While the pathologic findings in brains of cocaine users have not been well studied, both multiple sclerosis and progressive multifocal leukoencephalopathy are associated with marked gliosis.

A second possibility for the increased CR peak may be increased glial content, as discussed above in association with increased levels of MI. The concomitant increase in CR along with MI levels is supported by a significant correlation of CR and MI ( $r=0.77$ ;  $P=.002$ ) in the temporoparietal cortex. The same study that showed MI

to be a glial-specific marker also demonstrated that phosphocreatine/adenosine triphosphate is 3 times higher in glial cells than in neurons.<sup>36</sup> Another study showed that MRS of cell cultures of astrocytes have 2 times higher CR and 3 times higher taurine concentrations than neurons.<sup>54</sup> Because of the proximity of taurine (at 3.08 ppm) to CR (at 3.04 ppm), the elevated CR peak in our study may, in fact, be caused partly by elevated taurine levels. However, whether the elevated CR peak results from actual elevation of CR compounds or of taurine, both explanations support the increase in glial content. Therefore, increase in glial cells would elevate the total brain CR or taurine level, while a concomitant decrease in neuronal cells, and hence a decrease in NA and CR compounds, would lower the effect of the elevated CR level. However, because our patients with myotonic dystrophy had only minimal decreases in NA level, the changes in CR concentration appear to be related primarily to the increased glial content.

The increased CHO level observed in gray matter of the patients with myotonic dystrophy also supports possible increased glial content. Urenjak et al<sup>54</sup> showed in their cell culture studies that astrocytes and oligodendrocytes both have 2 to 3 times higher CHO levels than do neurons. In addition, elevated CHO level may be seen in conditions with cell membrane abnormalities or white matter diseases, such as multiple sclerosis, progressive multifocal leukoencephalopathy, and human immunodeficiency virus.<sup>41,42,52,55</sup> All of these diseases are associated with demyelination, gliosis, and increased oligodendrocyte activities in association with the repair processes. Our patients with myotonic dystrophy showed minimal to mild periventricular white matter hyperintensities. Although we did not examine the white matter directly in our localized MRS, we would expect an even higher elevation of CHO levels in these brain regions. Future MRS studies of myotonic dystrophy should include these white matter brain regions. Finally, patients with myotonic dystrophy show marked somatic mosaicism of the CTG repeat length<sup>56</sup>; therefore, the lack of correlations between CHO levels and CTG repeats from the blood cells might not reflect the actual contribution of CTG repeats to CHO metabolism in the brain tissue.

In summary, we found significant abnormalities of cerebral metabolites with <sup>1</sup>H MRS in patients with myotonic dystrophy. The abnormalities were more pronounced in the midoccipital gray matter than in the temporoparietal region. The most significant abnormalities were increased concentrations of MI and CR, which were associated with the longer CTG repeats. We attribute these abnormalities to increased glial content in the 2 brain regions studied. This study shows that <sup>1</sup>H MRS is a powerful tool to evaluate brain biochemistry, which may reflect the extent of the pathological involvement in the brains of patients with myotonic dystrophy. Future studies correlating MRS, CTG repeats, and neuropathological findings will be necessary to confirm these relationships.

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## REFERENCES

- Johnson K, Shelbourne P, Davies J, et al. A new polymorphic probe which defines the region of chromosome 19 containing the myotonic dystrophy locus. *Am J Hum Genet.* 1990;46:1073-1081.
- Harley H, Brook J, Floyd J, et al. Detection of linkage disequilibrium between the myotonic dystrophy locus and a new polymorphic DNA marker. *Am J Hum Genet.* 1991;49:68-75.
- Buxton J, Shelbourne P, Davies J, et al. Detection of an unstable fragment of DNA specific to individuals with myotonic dystrophy. *Nature.* 1992;355:547-548.
- Fu Y, Pizzuti R, Fenwick J, et al. An unstable triplet repeat in a gene related to myotonic dystrophy. *Science.* 1992;255:1256-1258.
- Jansen G, Mahadevan M, Amemiya C, et al. Characterization of the myotonic dystrophy region predicts multiple protein isoform-encoding mRNAs. *Nat Genet.* 1992;1:261-266.
- Klesert T, Otten A, Bird T, Tapscott S. Trinucleotide repeat expansion at the myotonic dystrophy locus reduces expression of DMAHP. *Nat Genet.* 1997;16:402-406.
- Thornton C, Wymer J, Simmons Z, McClain C, Moxley R. Expansion of the myotonic dystrophy CTG repeat reduces expression of the flanking DMAHP gene. *Nat Genet.* 1997;16:407-409.
- Harper P. *Myotonic Dystrophy.* 2nd ed. London, England: WB Saunders Co; 1989.
- Calderon R. Myotonic dystrophy: a neglected cause of mental retardation. *J Pediatr.* 1966;68:423-431.
- Bird T, Follett C, Griep E. Cognitive and personality function in myotonic muscular dystrophy. *J Neurol Neurosurg Psychiatry.* 1983;46:971-980.
- Woodward J, Heaton R, Simon D, Ringel S. Neuropsychological findings in myotonic dystrophy. *J Clin Neuropsychol.* 1982;4:335-342.
- Stuss D, Kates M, Poirier C, et al. Evaluation of information-processing speed and neuropsychological functioning in patients with myotonic dystrophy. *J Clin Exp Neuropsychol.* 1987;9:131-146.
- Huber S, Kissel J, Shuttleworth E, Chakeres D, Clapp L, Brogan M. Magnetic resonance imaging and clinical correlates of intellectual impairment in myotonic dystrophy. *Arch Neurol.* 1989;46:536-540.
- Palmer B, Boone K, Chang L, Lee A, Black S. Cognitive deficits and personality patterns in maternally versus paternally inherited myotonic dystrophy. *J Clin Exp Neuropsychol.* 1994;16:784-795.
- Brumback R. Disturbed personality and psychological adjustment in myotonic dystrophy: relationship to intellectual/cognitive function and underlying affective disorder (depression). *Psychol Rep.* 1987;60:783-796.
- Malloy P, Mishra S, Adler S. Neuropsychological deficits in myotonic muscular dystrophy. *J Neurol Neurosurg Psychiatry.* 1990;53:1011-1013.
- Censori B, Danni M, Del Pesce M, Provinciali L. Neuropsychological profile in myotonic dystrophy. *J Neurol.* 1990;237:251-256.
- Chang L, Anderson T, Migneco O, et al. Cerebral abnormalities in myotonic dystrophy: cerebral blood flow, magnetic resonance imaging, and neuropsychological tests. *Arch Neurol.* 1993;50:917-923.
- Glantz R, Wright R, Huckman M, Garron D, Siegel I. Central nervous system magnetic resonance imaging findings in myotonic dystrophy. *Arch Neurol.* 1988;45:36-37.
- Damian M, Schilling G, Bachmann G, Simon C, Stoppler S, Dorndorf W. White matter lesions and cognitive deficits: relevance of lesion pattern? *Acta Neurol Scand.* 1994;90:430-436.
- Censori B, Provinciali L, Danni M, et al. Brain involvement in myotonic dystrophy: MRI features and their relationship to clinical and cognitive conditions. *Acta Neurol Scand.* 1994;90:211-217.
- Fiorelli M, Duboc D, Mazoyer B. Decreased cerebral glucose utilization in myotonic dystrophy. *Neurology.* 1992;50:91-94.
- Yoshimura N, Otake M, Igarashi K, Matsunaga M, Takebe K, Kudo H. Topography of Alzheimer's neurofibrillary change distribution in myotonic dystrophy. *Clin Neuropathol.* 1990;9:234-239.
- Kiuchi A, Otsuka N, Namba Y, Nakano I, Tomonaga M. Presenile appearance of abundant Alzheimer's neurofibrillary tangles without senile plaques in the brain in myotonic dystrophy. *Acta Neuropathol.* 1991;82:1-5.
- Vermersch P, Sergeant N, Ruchoux M, et al. Specific tau-variants in the brains of patients with myotonic dystrophy. *Neurology.* 1996;47:711-717.
- Culebras A, Segarra J, Feldman R. Cytoplasmic inclusion bodies within neurons of the thalamus in myotonic dystrophy: a light and electron microscope study. *J Neurol Sci.* 1973;19:319-329.
- Folstein M, Folstein S, McHugh P. Mini-Mental State: a practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res.* 1975;12:189-198.
- Brook J, McCurrach M, Harley H, et al. Molecular basis of myotonic dystrophy: expansion of a trinucleotide (CTG) repeat at the 3' end of a transcript encoding a protein kinase family member. *Cell.* 1992;68:799-808.
- Harley H, Brook J, Rundle S, et al. Expansion of an unstable DNA region and phenotypic variation in myotonic dystrophy. *Nature.* 1992;355:545-546.
- Bottomley PA. Spatial localization in NMR spectroscopy in vivo. *Ann N Y Acad Sci.* 1987;508:333-348.
- Ernst T, Chang L. Elimination of artifacts in short echo time <sup>1</sup>H MR spectroscopy of the frontal lobe. *Magn Reson Med.* 1996;36:462-468.
- Ernst T, Kreis R, Ross BD. Absolute quantitation of water and metabolites in the human brain, I: compartments and water. *J Magn Reson.* 1993;B102:1-8.
- Kreis R, Ernst T, Ross BD. Absolute quantitation of water and metabolites in the human brain, II: metabolite concentrations. *J Magn Reson.* 1993;B102:9-19.
- Damian M, Bachmann G, Herrmann D, Dorndorf W. Magnetic resonance imaging of muscle and brain in myotonic dystrophy. *J Neurol.* 1993;240:8-12.
- Hashimoto T, Tayama M, Yoshimoto T, et al. Proton magnetic resonance spectroscopy of brain in congenital myotonic dystrophy. *Pediatr Neurol.* 1995;12:335-340.
- Brand A, Richter-Landsberg C, Leibfritz D. Multinuclear NMR studies on the energy metabolism of glial and neuronal cells. *Dev Neurosci.* 1993;15:289-298.
- Redman J, Fenwick RJ, Fu H, Pizzuti A, Caskey C. Relationship between parental trinucleotide GCT repeat length and severity of myotonic dystrophy in offspring. *JAMA.* 1993;269:1960-1965.
- Novelli G, Gennarelli M, Menegazzo E, et al. (CTG)<sub>n</sub> triplet mutation and phenotypic manifestations in myotonic dystrophy patients. *Biochem Med Metab Biol.* 1993;50:85-92.
- Bruhn H, Frahm J, Merboldt K, et al. Multiple sclerosis in children: cerebral metabolic alterations monitored by localized proton magnetic resonance spectroscopy in vivo. *Ann Neurol.* 1992;32:140-150.
- Chang L. In vivo magnetic resonance spectroscopy in HIV and HIV-related brain diseases. *Rev Neurosci.* 1995;6:365-378.
- Laubenberger J, Haussinger D, Bayer S, et al. HIV-related metabolic abnormalities in the brain: depiction with proton MR spectroscopy with short echo times. *Radiology.* 1996;199:805-810.
- Chang L, Ernst T, Tornatore C, et al. Metabolite abnormalities in progressive multifocal leukoencephalopathy by proton magnetic resonance spectroscopy. *Neurology.* 1997;48:836-845.
- Moats R, Ernst T, Shonk T, Ross BD. Abnormal cerebral metabolite concentrations in patients with probable Alzheimer's disease. *Magn Reson Med.* 1994;32:110-115.
- Ernst T, Chang L, Melchor R, Mehringer CM. Frontotemporal dementia and early Alzheimer disease: differentiation with frontal lobe H-1 MR spectroscopy. *Radiology.* 1997;203:829-836.
- Lien Y, Shapiro J, Chan L. Effects of hypernatremia on organic brain osmoles. *J Clin Invest.* 1990;85:1427-1435.
- Kimelberg H, O'Connor E, Kettenmann H. Effects of swelling on glial cell function. In: Lang F, Haussinger D, eds. *Interactions in Cell Volume and Cell Function.* Heidelberg, Germany: Springer-Verlag; 1993:158-186.
- Ono S, Inoue K, Mannen T, Kanda F, Jinnai K. Neuropathological changes of the brain in myotonic dystrophy: some new observations. *J Neurol Sci.* 1987;81:301-320.
- Nash S, Giros B, Kingsmore S, et al. Cloning, pharmacological characterization, and genomic localization of the human creatine transporter. *Recept Channels.* 1994;2:165-174.
- Rojas C, Wang J, Schwartz L, Hoffman E, Powell B, Brown R. A methionine to valine mutation in the skeletal muscle sodium channel alpha-subunit in human hyperkalemic periodic paralysis. *Nature.* 1991;354:387-389.
- McClatchey A, Van den Bergh P, Pericak-Vance M, et al. Temperature-sensitive mutations in the III-IV cytoplasmic loop region of the skeletal muscle sodium channel gene in paramyotonia congenita. *Cell.* 1992;68:769-774.
- Koch M, Steinmeyer K, Lorenz C, et al. The skeletal muscle chloride channel in dominant and recessive human myotonia. *Science.* 1992;257:797-800.
- Husted C, Goodin D, Hugg J, et al. Biochemical alterations in multiple sclerosis lesions and normal-appearing white matter detected by in vivo <sup>31</sup>P and <sup>1</sup>H spectroscopic imaging. *Ann Neurol.* 1994;36:157-165.
- Chang L, Ernst T, Strickland T. Neurochemical abnormalities and gender effects in abstinent asymptomatic cocaine users. In: Proceedings of the International Society for Magnetic Resonance in Medicine; April 27-May 3 1996; New York, NY. Abstract 992.
- Urenjak J, Williams S, Gadian D, Noble M. Proton nuclear magnetic resonance spectroscopy unambiguously identifies different neural cell types. *J Neurosci.* 1993;13:981-989.
- Tracey I, Carr C, Guimaraes A, Worth J, Navia B, Gonzalez R. Brain choline-containing compounds are elevated in HIV-positive patients before the onset of AIDS dementia complex: a proton magnetic resonance spectroscopic study. *Neurology.* 1996;46:783-788.
- Thornton C, Johnson K, Moxley RT. Myotonic dystrophy patients have larger CTG expansions in skeletal muscle than in leukocytes. *Ann Neurol.* 1994;35:104-107.