Seven-Tesla Proton Magnetic Resonance Spectroscopic Imaging in Adult X-Linked Adrenoleukodystrophy

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Background: Adults with X-linked adrenoleukodystrophy (X-ALD) remain at risk for progressive neurological deterioration. Phenotypes vary in their pathology, ranging from axonal degeneration to inflammatory demyelination. The severity of symptoms is poorly explained by conventional imaging.

Objective: To test the hypothesis that neurochemistry in normal-appearing brains differs in adult phenotypes of X-ALD and that neurochemical changes correlate with the severity of symptoms.

Patients and Methods: Using a 7-Tesla scanner, we performed structural and proton magnetic resonance spectroscopic imaging in 13 adult patients with X-ALD: 4 patients with adult cerebral ALD, 5 patients with adrenomyeloneuropathy (AMN), and 4 female heterozygotes. Nine healthy controls were included.

Results: Among adult X-ALD phenotypes, the myo-inositol to creatine ratio was 46% higher and the choline to creatine ratio was 21% higher in normal-appearing white matter of those with adult cerebral ALD compared with those with AMN (P <.05). Both N-acetylaspartate to creatine (P = .03) and glutamate to creatine (P = .04) ratios were lower in AMN patients than in controls. There were no significant differences between patients with AMN and female heterozygotes. In the cortex, patients with adult cerebral ALD had lower N-acetylaspartate to creatine ratios compared with female heterozygotes and controls (P = .02). The global myo-inositol to creatine ratio demonstrated a significant association with Expanded Disability Status Scale score (Spearman ρ = 0.66, P = .04).

Conclusions: Seven-Tesla proton magnetic resonance spectroscopic imaging reveals differences in the neurochemistry of adult cerebral ALD but cannot distinguish AMN patients from female heterozygotes. Myo-inositol to creatine ratio correlates with the severity of the symptoms and may be a meaningful biomarker in adult X-ALD.

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esized that proton magnetic resonance spectroscopic imaging (MRSI) using a 7-T scanner could differentiate among adult X-ALD phenotypes and that neurochemical changes would correlate with the severity of symptoms.

**METHODS**

**PARTICIPANTS**

Participants were recruited from the leukodystrophy clinic at Massachusetts General Hospital. Thirteen adult patients with the biochemical defect for ALD and 9 healthy controls were enrolled in this study (Table). The patients had elevated concentrations of VLCFA in plasma, confirming the diagnosis of X-ALD. Neurologic examinations consisted of the Expanded Disability Status Scale (EDSS). A neurologist blinded to the patients’ spectroscopy results (G.O.) performed the EDSS. The local institutional review board approved the study, and informed consent was obtained from each participant.

**1.5-T MRSI PROTOCOL**

The clinical standard protocol for ALD patients on the Signa 1.5-T scanner using a quadrature head coil (General Electric, Milwaukee, Wisconsin) included an axial T1-weighted image sequence with the following parameters: 220-mm field of view, minimum echo time, 450 milliseconds of repetition time, and 3-mm skip 1-mm slice thickness. This resulted in a resolution of 0.86 x 0.86 x 5 mm. Magnetic resonance spectroscopic imaging was performed using a point-resolved spectroscopy (PRESS) sequence with chemical shift selective water suppression on a field of view of 220 mm and 16 x 16 phase-encoding steps, which were subsequently interpolated to 32 x 32 and an echo time/repetition time of 144 ms/1500 ms. In addition, 6 spatial outer-volume suppression pulses were used for improved fat suppression.

**7-T MRSI PROTOCOL**

Magnetic resonance structural imaging and spectroscopic imaging experiments were performed on a 7-T scanner (Siemens AG, Erlangen, Germany) using a detunable bird cage coil for excitation and an 8-channel coil array for signal reception. The imaging examination included sagittal T1-weighted images (field of view, 220 mm; echo time/repetition time, 5 ms/30 ms; slice thickness, 2.5 mm) and axial magnetization-prepared rapid gradient-echo (MPRAGE) images (echo time/repetition time/inversion time = 3.6 ms/2500 ms/1100 ms; 120 contiguous slices) with a resolution of 0.56 x 0.56 x 1 mm (0.31 mm3) from which the volume of interest for MRSI was prescribed.

Two-dimensional proton MRSI spectra were acquired in a 1.5-cm thick oblique-axial slice parallel to a line through the anterior and posterior commissures at the levels of the white matter centrum semiovale as well as the cingulate cortex. The volume of interest was selectively excited using PRESS (echo time/repetition time = 35 ms 2 s) with water suppression enhanced through T1 effects and 6 spatial outer-volume suppression pulses for improved fat suppression. The field of view (20 cm) was partitioned into 16 x 16 phase-encoding steps, resulting in a nominal voxel size of 1.25 x 1.2 x 1.5 cm (2.5 cm3) and a scan time of 8 minutes. Other MRSI acquisition parameters included a bandwidth of 4000 Hz and a water-suppression bandwidth of 120 Hz. Before data acquisition, the volume of interest underwent an automatic shim routine using first- and second-order shimming, followed by an automatic manual shimming adjustments. The typical line width (full-width at half maximum) for water within the entire volume of interest was 26 to 30 Hz, yielding a spectral resolution of 9 Hz for Cr.

**DATA PROCESSING**

Spectra were processed using an automated parametric spectral analysis method, LCModel, 6.01,14 which seeks to determine the optimum parameters that enable so-called model functions to best describe the data to determine signal intensities of brain metabolites NAA, Cr, Cho, Glu, and MI. The basis set or model functions to analyze the metabolites via LCModel was generated by GAMMA (ETH Zürich, Zurich, Switzerland), a program designed to simulate magnetic resonance spin systems. Only voxels identified by visual inspection on an overlay of structural MPRAGE and spectral grids as predominantly gray matter or predominantly white matter were included in the metabolic analysis. Voxels were excluded from the analysis owing to poor water and fat suppression determined by visual inspection of spectral data and if their location was close to the PRESS-box boundary in regions of excitation chemical shift artifact. Metabolic concentrations were only used in the analysis if their standard error estimates were less than 15%. In particular, concentrations of Glu were only included in the analysis if both glutamine and Glu met that criteria.

**STATISTICAL ANALYSIS**

A multivariate analysis of variance (ANOVA) was performed to test our hypothesis that the metabolic ratios (NAA/Cr, Cho/Cr, Glu/Cr, and MI/Cr) in each of 2 tissue types, gray matter and normal-appearing white matter (NAWM), are altered in different phenotypes (controls, ACALD, AMN, and female heterozygotes). Analyses of variance for individual metabolites across phenotypes were carried out only if the multivariate ANOVA was significant (P < .05). As overall α was limited by the initial multivariate ANOVA, no corrections for multiple comparisons were needed. If the ANOVA was significant (P < .05) or showed a trend toward significance (P < .1), a Holm t test was performed to test for differences in metabolic ratios among the phenotypes. Associations between various global metabolic ratios, defined as the metabolic ratio of all gray and white matter voxels combined per patient, and EDSS scores were assessed using the Spearman rank correlation. In this context,
the effect of individual scale components of the EDSS (motor, sensory, bladder, and mental) was also examined. We further examined associations between plasma VLCFAs, in particular hexacosanoic acid (C26:0), and global metabolic ratios.

RESULTS

Clinical characteristics are listed in the Table. Mean EDSS scores were 3.3 (standard deviation [SD], 1.9; range, 1.5-6), 4.5 (SD, 3.1; range, 1-8), and 2.8 (SD, 0.9; range, 2-4) for ACALD patients, AMN patients, and female heterozygotes, respectively.

All 4 ACALD patients had characteristic T1 hypointense lesions in the splenium of the corpus callosum on MPRAGE images (Figure 1). Two of the 4 AMN patients had T1 hypointense lesions confined to the bilateral corticospinal tracts. The remaining 2 AMN patients did not have and none of the 4 female heterozygotes and none of the controls had brain abnormalities on conventional sequences. In contrast to routine 1.5-T images, MPRAGE at 7 T showed high resolution of individual fiber tracts (resolution, 0.56 × 0.56 × 1 mm).

All MRSI voxels were assigned to either predominantly white matter or predominantly gray matter based on visual inspection by a neuroradiologist. On average, 12 voxels (SD, 5 voxels) were assigned to predominantly white matter and 5 voxels (SD, 2 voxels) were assigned to predominantly gray matter. In addition, in 3 ACALD patients, a mean of 5 voxels (SD, 1) were assigned to lesions. Peaks of NAA, Cho, Cr, MI, and Glu levels were detected in all patients and controls, and mean values for metabolic ratios within NAWM and gray matter were determined and are shown in the bar graphs in Figure 2 and Figure 3. There was no significant age difference between the 13 ALD patients (mean, 37 years; SD, 13 years) and the 9 controls (mean, 32 years; SD, 8 years) (P=.3). In neither our control nor our X-ALD population did we find any significant correlations between age and metabolic ratios.

METABOLIC RATIOS IN WHITE MATTER

Initially, multivariate ANOVA was performed using a model that included 4 metabolic ratios, NAA/Cr, Cho/Cr, Cho/Cr, and so on.
Glu/Cr, and MI/Cr, in 2 tissue types, gray matter and NAWM. There was a significant difference between ACALD patients, AMN patients, female heterozygotes, and controls (P = .01). Based on these results, we analyzed the individual 4 metabolic ratios in the 2 regions using ANOVA and the Holm t test to isolate differences between the phenotypes (Figure 2).

There were significantly decreased NAA/Cr ratios in the NAWM of AMN (P = .03) and ACALD (P = .03) patients compared with controls, but not in female heterozygotes. By contrast, Cho/Cr (P = .01) and MI/Cr (P = .02) ratios were increased in ACALD compared with controls, but not in AMN patients or female heterozygotes. In all X-ALD patients, including female heterozygotes, white matter Glu/Cr ratios showed a trend to be lower compared with those in controls (P = .09).

Among adult X-ALD phenotypes, MI/Cr ratios were 46% higher in ACALD compared with AMN patients (P = .02) and female heterozygotes (P = .03). The Cho/Cr ratio was 21% higher in ACALD compared with AMN patients (P = .06). There was a trend for NAA/Cr ratios to be lower in ACALD compared with AMN patients and female heterozygotes, though this was not significant. There were no significant differences between AMN patients and female heterozygotes.

**METABOLIC RATIOS IN GRAY MATTER**

There were decreased NAA/Cr ratios in cortical gray matter (P = .02) (Figure 3) in male X-ALD patients compared with controls. There was no significant elevation of the Cho/Cr, Glu/Cr, or MI/Cr ratios in the cortex of X-ALD patients compared with healthy volunteers. Among adult X-ALD patients, ACALD patients had lower NAA/Cr ratios than female heterozygotes (P = .02). There were no significant differences between AMN and ACALD patients or between AMN patients and female heterozygotes.

A significant correlation between NAA/Cr and Glu/Cr ratios (Spearman ρ = 0.66, P = .002) was found. The NAA/Cr and MI/Cr ratios (Spearman ρ = −0.49, P = .03)
as well as the Glu/Cr and MI/Cr ratios (Spearman $\rho=-0.55$, $P=.02$) showed a significant negative correlation.

**ASSOCIATION WITH CLINICAL DISABILITY AND PLASMA VLCFA**

The scatterplot matrix in Figure 4 demonstrates the interrelationship between the global metabolic estimates and clinical disability index (EDSS). The global metabolic ratio was defined as the metabolic ratio of all gray and white matter voxels combined per patient. The EDSS scores demonstrated a significant association with the global MI/Cr ratios (Spearman $\rho=0.66$, $P=.04$). Assessment of individual scale components revealed significant correlations between motor function and global metabolic ratios of Glu/Cr (Spearman $\rho=-0.64$, $P=.003$), MI/Cr (Spearman $\rho=0.67$, $P=.002$), and NAA/Cr (Spearman $\rho=-0.61$, $P=.002$). This correlation was also present for bladder function (Glu/Cr ratio, Spearman $\rho=-0.67$, $P=.002$; MI/Cr ratio, Spearman $\rho=0.67$, $P=.002$; NAA/Cr ratio, Spearman $\rho=-0.59$, $P=.002$) but not for sensory or mental function.

We found an inverse correlation between the patients' plasma VLCFA (absolute C26:0 levels) and their global NAA/Cr ratio (Spearman $\rho=-0.67$, $P=.02$) (Figure 4). On the other hand, we do not find any correlations between C26:0 and any of the other metabolic markers (MI/Cr and C26:0, Spearman $\rho=0.36$, $P=.38$; Cho/Cr and C26:0, Spearman $\rho=0.11$, $P=.7$; Glu/Cr and C26:0, Spearman $\rho=-0.52$, $P=.18$).

**COMMENT**

Proton magnetic resonance spectroscopic imaging at 7 T was used to study adult patients with X-ALD. Metabolic ratios of NAA/Cr, Cho/Cr, Glu/Cr, and MI/Cr were analyzed in both white and gray matter. We hypothesized that adult phenotypes with X-ALD would differ in their neurochemistry, and we have found that among adult X-ALD phenotypes, MI/Cr ratios were 46% higher and Cho/Cr were 21% higher in NAWM of ACALD than in AMN ($P<.05$). There were no significant differences between AMN patients and female heterozygotes.
Traditional methods to distinguish adult phenotypes of X-ALD include clinical and biochemical assessment as well as imaging at 1.5 T. However, these methods remain inadequate to explain the dramatic differences in pathology and symptom severity among ALD patients. We demonstrate that magnetic resonance imaging at 7 T allows for better visualization of X-ALD lesion architecture, white matter tracts, and gray-white matter distinction compared with 1.5 T (Figure 1). Improved signal-to-noise ratio and chemical-shift dispersion result in better spectral resolution and more reliable detection of metabolites, such as Glu and MI.

The best correlation with the EDSS, a validated clinical rating scale, was with the MI/Cr ratio (Figure 4). Because microglia produce MI and ALD protein is highly expressed in glial cells, the increased MI level may reflect microglial activation and gliosis. Our findings of elevated MI and Cho, the latter an established marker of cell membrane turnover and demyelination, further support this notion.

Consistent with prior reports, our results show that NAA was decreased in NAWM of all adult X-ALD phenotypes, suggesting a tight link between the mutated peroxisome and axonal dysfunction. Interestingly, higher plasma VLCFA levels were associated with a lower NAA/Cr ratio. Recent findings in a conditional knockout mouse suggest that the degree of peroxisomal dysfunction in oligodendrocytes alone may regulate the NAA was decreased in NAWM of all adult X-ALD patients, suggesting that the normal X chromosome may protect against the inflammatory brain disease but not the noninflammatory axonopathy. Prior reports have shown that NAA levels are reduced within the corticospinal tract fibers, suggesting axonal dysfunction. We found a similar trend, though this did not reach significance. Of note, we did not find decreases in NAA/Cr ratios in gray matter, as we found in males with X-ALD.

Despite the advantages at 7 T, the interpretation of 7-T MRSI data sets requires care. Voxels closest to the scalp were necessarily excluded owing to poor water and lipid suppression as determined by visual inspection of spectra along the edge of the PRESS volume. Furthermore, the quantification of spectral data in the presence of substantial radiofrequency excitation field (BI) variations is a challenging task, and in this study we relied on ratios of metabolites to Cr, which, though improving robustness for conditions in which the Cr signal is stable, limits the interpretation of the findings in the presence of unknown Cr variations. In childhood ALD, high concentrations of Cr have been reported in brain lesions but not in NAWM, the subject of our study.

This study demonstrates the feasibility of proton MRSI at 7 T and takes advantage of the improved signal dispersion and higher signal-to-noise ratio of 7 T compared with those of a lower field. Limitations of our study are the small sample size as well as the limited spatial coverage of regions of interest, which we are addressing with full-brain spiral spectroscopic imaging. The expanded coverage of such methods, combined with segmented high-resolution structural images, will allow us to analyze the tract-specific nature of metabolic abnormalities in X-ALD and obtain improved quality cortical data.

The utility of the MI/Cr ratio as a biomarker in adult X-ALD will require further investigation. Specifically, to determine its disease specificity and predictive ability with early diagnosis of ALD, a study that prospectively fol-
lows male hemizygotes with normal magnetic resonance imaging results is needed. How the degree of VLCFA accumulation in plasma contributes to the neurochemical changes in various phenotypes is currently unknown. A better understanding of the regional metabolic impact of VLCFA in the brains of various phenotypes will help elucidate this question. Hence, a longitudinal study with sequential 7-T proton MR examinations will be necessary to establish the sensitivity of the M1/Cr ratio in predicting disease progression.

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