

## Proton MR Spectroscopy in Lesch-Nyhan Disease

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**Summary:** In vivo proton spectra for four patients with Lesch-Nyhan disease and four control subjects matched for age and sex were acquired from voxels ( $1.5 \times 1.5 \times 1.5 \text{ cm}^3$ ) placed in the prefrontal cortex and striatum. The patients with Lesch-Nyhan disease had decreased metabolites, especially *N*-acetylaspartate and glutamate/glutamine, only in the prefrontal cortex as compared with the control group. These findings suggest axonal loss in the prefrontal area of this population. The cortical glutamate/glutamine peak decrement does not confirm cytopathologic studies of Lesch-Nyhan disease and deserves further investigation.

Lesch-Nyhan disease is an X-linked recessive disorder of purine metabolism associated with a deficiency of hypoxanthine-guanine phosphoribosyltransferase (HPRT), an enzyme that catalyzes the conversion of guanine and hypoxanthine to their purine nucleotides, guanylic acid and inosinic acid (1), building blocks of DNA and RNA. In the absence of HPRT (and its salvage pathways), there is excessive synthesis of purine nucleotides, resulting in overproduction of uric acid, uricosuria, gouty arthritis, nephropathy (2), neurologic manifestations (3) such as athetosis and self-injurious behavior (SIB). Dopaminergic (4-7) and serotonergic (8, 9) mechanisms have been postulated as contributors to SIB in Lesch-Nyhan disease. Studies evaluating the susceptibility of 6-hydroxydopamine-lesioned rats for SIB after microinjection with muscimol into the substantia nigra have also suggested a possible role of  $\gamma$ -aminobutyric acid (GABA) receptor sensitivity in SIB (10) and of glutamate neurotransmitter systems (11, 12). Animal studies and positron emission tomographic studies in patients with Lesch-Nyhan disease (13, 14) suggest that the striatum may play a prominent role. The purpose of our project was to characterize the metabolite profile in the striatal and prefrontal cortex in four patients with Lesch-Nyhan disease by using in vivo proton MR spectroscopy. The orbitomedial prefrontal location was chosen because of the axonal connections with the ventromedial striatum. This area

has been shown to be deficient in dopamine transport binding (7), suggestive of dopaminergic nerve terminal loss (6) in patients with this disorder.

### Methods

The subjects consisted of four male patients (ages 6, 16, 17, and 22 years old) with Lesch-Nyhan disease. The study was approved by the Human Subjects Protection Committee, and informed consent was obtained from all patients or their parents or legal guardians. Each patient had HPRT levels less than 1% of normal values, measured in erythrocytes or fibroblasts. The degree of SIB and aggressive/destructive behavior was ascertained with the Aberrant Behavior Checklist (15) and the Behavior Problems Inventory (16). Treatment with allopurinol was continued throughout the imaging procedure in all patients. Three patients continued to take diazepam, metoclopramide hydrochloride, and propranolol, respectively.

The control subjects consisted of four healthy volunteers, matched for age and sex, who were screened by a board-certified psychiatrist to rule out DSM-IV axis I psychiatric diagnoses, a history of acute or chronic medical-neurologic illness, head trauma, or substance abuse.

#### *Proton MR Spectroscopic Data Acquisition*

All subjects were sedated with propofol during the procedure, except for subject 1 who was sedated with pentobarbital according to standard protocol. A bolus of 1.2 to 2.0 mg of propofol per kilogram was administered intravenously by an anesthesiologist for induction of sedation 15 minutes before the imaging. Maintenance levels were achieved with continuous intravenous infusion rates of 60 to 160  $\mu\text{g}/\text{kg}$  per minute. All subjects were monitored with oximetry during the scanning.

Axial and coronal localizing series were acquired for the selected region. Spin-echo MR imaging was performed with parameters of 500 ms/8 ms/1 (TR/TE/excitations), 3-mm sections interleaved, a  $256 \times 192$  matrix, and a 24-cm field of view. In vivo proton MR spectra were acquired using the standard quadrature bird cage head coil (17). Two  $3.4 \text{ cm}^3$  voxels ( $2 \times 2 \times 2 \text{ cm}^3$ ) containing as little CSF or white matter as possible were chosen in the striatum (ie, head of the caudate nucleus and putamen) and orbitomedial prefrontal cortex (Fig 1) using a stimulated Echo Acquisition Mode sequence with TR/TE of 1500 ms/20 ms. Water suppression was achieved using the chemical-shift selective sequence (a combination of three frequency-selective radio-frequency pulses followed by dephasing

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$B_0$ -gradient crusher pulses, each radio-frequency pulse selective on water frequency).

*Proton MR Spectroscopic Data Processing*

A commercially available package was used for data processing, which was performed in the following steps: 1) low-frequency filtering of free induction decay; 2) 0.5-Hz Lorentz-Gauss transformation, fast Fourier transformation, and phase correction; 3) line fitting and spectral integration; and 4) comparison with control spectra. The following assignments (18) were made: *N*-acetylaspartate (NAA), 2 ppm, 2.5 ppm; glutamine/glutamate, 2.2 ppm, 3.6 ppm; GABA, 1.85 ppm, 2.85 ppm, 2.4 ppm; creatine, 3 ppm, 3.9 ppm; choline, 3.2 ppm; *myo*-inositol, 3.5 ppm, 4 ppm; glucose, 3.35 ppm, 3.75 ppm; and lipids (fat), 0.8 to 1.5 ppm. The average squared difference between the fitted data and the actual data for areas under the curve was calculated for each spectrum. All results were ex-

pressed as ratios of creatine (Fig 2). Creatine was used as a control because its peak has been shown to be stable in subjects with a range of disorders (19).

**Results**

Statistical significance of differences between group means for proton MR spectroscopic metabolites was calculated using two-tailed *t*-tests with an assumption of unequal variance. *P* values for *myo*-inositol ( $P = .21$ ;  $P = .98$ ), choline moieties ( $P = .30$ ;  $P = .69$ ), glutamate/glutamine ( $P = .17$ ;  $P = .94$ ), and NAA ( $P = .38$ ;  $P = .87$ ) were not significant for the prefrontal or the left caudate nucleus regions of interest, respectively.

All patients had (nonsignificantly) decreased prefrontal metabolite spectral peaks as compared with control subjects (Fig 2). Spectral ratios in the left caudate nucleus did not vary between patients and control subjects. In addition, we estimated the effective size of the prefrontal signals necessary to characterize the magnitude of differences between group means. For the prefrontal region, effect size estimates were 0.71 for NAA, 0.80 for choline, 0.98 for *myo*-inositol, 1.19 for glutamate, and undefined for creatine, since each peak was scaled to the height of the creatine peak.

**Discussion**

In this preliminary study there appeared to be a decrease of spectral metabolites, especially NAA and glutamate/glutamine peaks in the prefrontal cortex but not in the striatum of patients with Lesch-Nyhan disease. Our results support a previous cytopathologic study showing little or no variation in brain amino acids in the basal ganglia of three patients with Lesch-Nyhan disease (20). The same study showed glutamine and glutamate increase in the limbic cortical

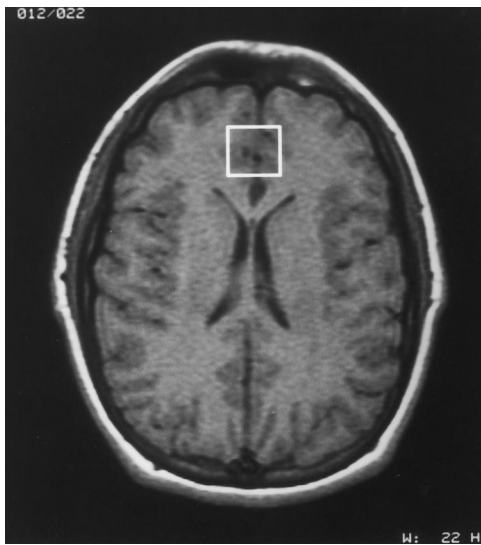


FIG 1. Axial MR image shows  $2 \times 2 \times 2 \text{ cm}^3$  voxel placed in the orbitomedial prefrontal cortex of a patient with Lesch-Nyhan disease.

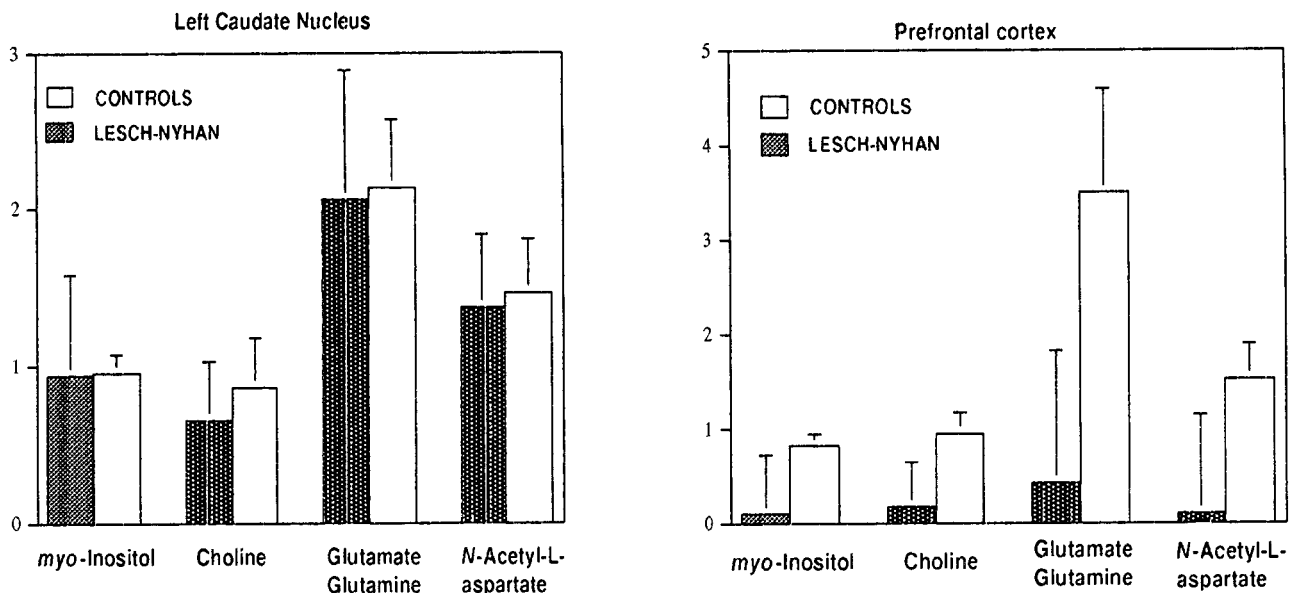


FIG 2. Proton MR spectroscopic mean metabolite/creatine ratios show a decrease in NAA and glutamate/glutamine peaks in prefrontal cortex but not in striatum of four patients with Lesch-Nyhan disease as compared with healthy control subjects.

area (20), a finding not supported by our data in the orbitomedial prefrontal cortex. Our findings suggest a functional deficit of glutamate/glutamine and NAA in the prefrontal cortex of persons with Lesch-Nyhan disease as compared with control subjects.

These findings must be interpreted with caution owing to the limited specificity of in vivo proton MR spectroscopy. A pattern of coupled resonances between 2.1 and 2.5 ppm are assigned to both glutamate and glutamine (21), representing the combined signals from both molecules. Current in vivo proton MR spectroscopic resolution does not permit differentiation of glutamate and glutamine resonances or allow us to determine compartments of origin (ie, neuronal or glial or both). Our lack of statistical significance is due in part to the small size of this sample.

Studies showing diminished NAA in multiple sclerosis (22–24) (eg, a disease with axonal loss) support a view of NAA as a marker for decreased neuronal density (25). Our findings of prefrontal NAA reduction in patients with Lesch-Nyhan disease relative to that in control subjects suggest an axonal deficit in the orbitomedial prefrontal area of these patients. This supports hypotheses of developmental neuronal deficiencies in this population (6). One study that showed NAA as a regulator of protein synthesis (26) would suggest decreased protein catabolism as a possible mechanism. However, another study, in which [<sup>13</sup>C] leucine was used as a tracer, showed normal protein turnover in three children with Lesch-Nyhan disease (27).

### Conclusion

Our findings are suggestive of axonal loss in the prefrontal area of patients with Lesch-Nyhan disease. The prefrontal glutamate/glutamine peak decrement does not confirm cytopathologic studies of this disorder and deserves further investigation.

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