Late infantile neuronal ceroid lipofuscinosis (LINCL), a form of Batten disease, is an autosomal recessive lysosomal storage disease that results in neurodegeneration. It first manifests between the ages of 2–4 years through seizures followed by deteriorating motor, cognitive, and visual abilities, traditionally leading to death by ages 8–12 years. Definitive diagnosis of LINCL is done via genetic testing for mutations in the CLN2 gene, which results in deficiency of the lysosomal protease tripeptidyl peptidase-I. Absence of this protease leads to lysosomal distension and neuronal death. There are no known treatments for LINCL other than symptom management. A better understanding of the anatomic distribution of neuronal degeneration can be assessed by a disability scale based on speech, vision, language, and seizures. To monitor brain-directed therapies, a modified central nervous system (CNS) disability score has been established. However, these methods are nonspecific in that they do not implicate specific areas of the brain. Quantitative MR imaging techniques may supplement information provided by the disability scale and provide a more refined evaluation of neurodegeneration, as well as an opportunity for serial assessment of the same patient.

Diffusion-weighted MR imaging (DWI) is a technique that has been shown to detect water diffusion abnormalities in various disease states. Measurement of the apparent diffusion coefficient (ADC) provides a quantitative estimate of the restrictive nature of the motion of water molecules within tissue for each voxel in a diffusion-weighted image. The ADC is known to decrease with increasing age in normal children as the brain becomes increasingly myelinated and structured. This study used whole-brain ADC histograms to obtain a measure of the degree of water restriction in the entire brain. The whole-brain histogram was fitted using a dual Gaussian function in addition to a partial volume function. We hypothesized that the global maximum of the model fit characterizing the whole-brain ADC value derived by DWI techniques would supplement clinical disability scale information to provide a quantitative estimate of neurodegeneration, as well as disease progression and severity.

Materials and Methods

Patient Selection

The research protocol was reviewed and approved by the institutional review board at our institution. Eighteen patients presenting with genetically confirmed LINCL (9 boys and 9 girls) of ages 3.4 through 13.8 years participated in this protocol, for a total of 32 DWI examinations. The average age at diagnosis for all of the patients was 3.9 ± 1.0 years. Disease severity was clinically monitored throughout the study using a modified CNS disability scale. The visual component was removed from the scoring system while retaining measures...
Whole-brain ADC values for 18 patients with LINCL

| Patient | Age at Diagnosis, y | Scan | Age at Scan, y | CNS Score | ADC × 10⁻³ mm²/s |
|---------|---------------------|------|---------------|-----------|------------------|
| BD-01   | 5.3                 | 1    | 8.4           | 3         | 1.12             |
| BD-02   | 4.8                 | 3    | 6.6           | 3         | 1.18             |
| BD-03   | 3.5                 | 4    | 10.0          | 3         | 1.00             |
| BD-04   | 4.5                 | 6    | 6.9           | 3         | 0.96             |
| BD-05   | 4.8                 | 7    | 13.8          | 3         | 0.96             |
| BD-06   | 4.4                 | 8    | 6.2           | 5         | 0.96             |
| BD-07   | 3.3                 | 9    | 7.1           | 2         | 0.96             |
| BD-08   | 4.7                 | 10   | 8.0           | 3         | 1.10             |
| BD-09   | 3.3                 | 11   | 8.1           | 3         | 1.06             |
| BD-10   | 3.8                 | 12   | 6.6           | 3         | 1.02             |
| BD-11   | 4.4                 | 13   | 6.0           | 4         | 0.94             |
| BD-12   | 2.8                 | 14   | 7.0           | 3         | 0.98             |
| BD-13   | 4.3                 | 15   | 6.7           | 4         | 0.92             |
| BD-14   | 4.9                 | 16   | 7.7           | 3         | 0.98             |
| BD-15   | 1.9                 | 17   | 5.0           | 5         | 0.90             |
| BD-16   | 2.8                 | 18   | 5.4           | 5         | 0.90             |
| BD-17   | 4.4                 | 19   | 5.4           | 4         | 0.94             |
| BD-18   | 4.8                 | 20   | 4.4           | 4         | 0.92             |
| BD-19   | 4.2                 | 21   | 4.5           | 4         | 0.90             |
| BD-20   | 4.2                 | 22   | 4.4           | 6         | 0.84             |
| BD-21   | 1.9                 | 23   | 4.5           | 6         | 0.84             |
| BD-22   | 1.9                 | 24   | 3.4           | 5         | 0.90             |
| BD-23   | 2.8                 | 25   | 3.6           | 5         | 0.82             |
| BD-24   | 2.8                 | 26   | 4.7           | 4         | 0.88             |
| BD-25   | 4.8                 | 27   | 5.2           | 5         | 0.86             |
| BD-26   | 4.8                 | 28   | 5.3           | 5         | 0.90             |
| BD-27   | 2.8                 | 29   | 5.4           | 4         | 0.90             |
| BD-28   | 2.8                 | 30   | 3.4           | 5         | 0.84             |
| BD-29   | 4.4                 | 31   | 3.4           | 5         | 0.82             |
| BD-30   | 4.4                 | 32   | 3.4           | 5         | 0.84             |

Note:—ADC indicates apparent diffusion coefficient; LINCL, late infantile neuronal ceroid lipofuscinosis; CNS, central nervous system.

of motor function, seizure activity, and language skills to focus on the neurologic aspects of the disease.1 Each of these areas was ranked from 0 to 3 and then summed to provide a total score with 0 being the most severe. At the time of MR imaging, patients presented the following CNS disability scores: 2 (n = 1), 3 (n = 12), 4 (n = 6), 5 (n = 11), and 6 (n = 2).

Clinical MR Imaging Methods

All of the image data were acquired on a 3T MR imaging system (GE Medical Systems, Milwaukee, Wis). Conventional clinical imaging included T1-weighted, T2-weighted, and fluid-attenuated inversion recovery sequences. Next, a spin-echo diffusion-weighted echo-planar imaging sequence was implemented over the entire brain using a section thickness of 5 mm with an FOV of 22 cm, a matrix size of 128 × 128, a TR of 8.2 seconds, a TE of 70–80 ms, and 2 averages. Diffusion weighting was acquired using b = 1000 s/mm² in 3 orthogonal directions for a total scan time of 65 seconds.

Analysis Methods

Images were exported to an Optiplex Pentium 4 4.0-GHz PC (Dell, Round Rocker, Texas) and analyzed using the Interactive Data Language (IDL 6.2; ITT, Boulder, Colo). Images were masked to include voxels having a signal intensity greater than 15% of the maximum value in the DWI series. ADC values were calculated as follows:

\[
ADC = -\frac{1}{b} \ln \left( \frac{S}{S_0} \right)
\]

where \(S\) represents the signal intensity from the diffusion-weighted image and \(S_0\) the signal intensity without diffusion weighting (Fig 1). ADC values were placed in a normalized histogram of unit area. The normalized histogram was fitted with a dual Gaussian distribution function and a partial volume distribution function (Fig 2). These 3 functions were designed to segment the brain, CSF, and brain-CSF partial volume components, respectively. Modeling of these functions was based on arguments given previously8-10 but was modified in the following sense: the ADC distribution function was modeled as follows:

\[
P_{ADC} = f_{brain} p_{brain} + f_{CSF} p_{CSF} + (1 - f_{brain} - f_{CSF}) p_{PV}
\]

where

\[
p_{brain} = \frac{1}{\sqrt{2\pi \sigma_{brain}}} \exp \left[ -\frac{1}{2} \left( \frac{ADC - \mu_{brain}}{\sigma_{brain}} \right)^2 \right]
\]

and

\[
p_{CSF} = \frac{1}{\sqrt{2\pi \sigma_{CSF}}} \exp \left[ -\frac{1}{2} \left( \frac{ADC - \mu_{CSF}}{\sigma_{CSF}} \right)^2 \right]
\]

are the normalized Gaussian functions describing the distribution of ADC values in the brain and CSF, respectively, and \(f_{brain}\) are respective weighting factors. The partial volume distribution function was modeled under the assumption that voxels that consist of brain matter and CSF contain both components in all of the possible and equally probable fractions, that is:

\[
p_{PV} = \int_{0}^{1} \frac{1}{\sqrt{2\pi \sigma(t)}} \exp \left[ -\frac{1}{2} \left( \frac{ADC - \mu(t)}{\sigma(t)} \right)^2 \right] dt,
\]

where \(\mu(t) = (1-t)\mu_{brain} + t\mu_{CSF} \) and \(\sigma(t) = (1 - t)^2 \sigma_{brain}^2 + t^2 \sigma_{CSF}^2\). Curve fitting of the 6 parameters \(\mu_{brain}, \mu_{CSF}, \sigma_{brain}, \sigma_{CSF}, f_{brain}\) and \(f_{CSF}\) was performed within IDL 6.2 using a gradient expansion algorithm to compute a nonlinear least-squares fit to the data. Note that the partial volume function is completely determined by the parameters of the 2 Gaussians. The histogram bin containing the maximum value of the combined fitted function (Equation 2), of the cerebral compartment was used as a measure of the whole-brain ADC value.

A Monte Carlo algorithm was written in IDL 6.2 to verify the functional form of the equations describing the analytical partial volume model. Two standard normally distributed floating point pseudorandom number generators, \(r_{x,y}\), were used to sample points from

Fig 1. A, A diffusion-weighted image (b = 1000 s/mm²) and an image acquired without diffusion weighting (B) show enlarged sulci and dilated ventricles consistent with atrophic changes from a representative patient with LINCL.
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Results

The whole-brain ADC value containing both gray and white matter components of the brain was determined from the 32 DWI scans of the 18 LINCL subjects and given with age, CNS disability score at the time of the scan, and the age at diagnosis (Table). The estimate of percentage error on the maximum ADC value was 1.7% ± 0.3% (n = 5) using the statistical bootstrap method. The whole-brain ADC values derived from the histogram increased with age for all of the LINCL patients (Fig 4), in contrast with the decrease in ADC as a function of age as seen in age-matched control subjects. The ADC values increased linearly with patient age [ADC = 3.63 (±0.33) + 0.03 (±0.01) ].
The whole-brain ADC values were correlated with the disease duration calculated as the age at diagnosis subtracted from the age at examination. A linear trend showed significant correlation between the whole-brain ADC histogram values and disease duration yielding $r^2 = 0.68$ ($P < .0001$). There were no significant differences in the ADC values of the Gaussian representing brain parenchyma of the LINCL patients fitted using both Equation 2 and the triple-Gaussian methods. The point at which the lower 95% confidence interval of the LINCL patients crossed the upper 95% confidence interval of the control subjects was at 5 years. This implies that statistically significant deviations in whole-brain ADC may be detected as early as 5 years of age in this population.

A single-factor ANOVA test with a 95% confidence interval compared the mean of the whole-brain ADC histogram with the modified CNS disability scale. The ANOVA analysis yielded $F > F_{crit} (3.8 > 2.7)$ and a $P$ value of .01, confirming differences in the means of the whole-brain ADC values between patients grouped by LINCL scale. A linear regression confirmed that the whole-brain ADC values increased with disease severity ($r^2 = 0.27; P = .002$).

### Discussion

A number of noninvasive imaging approaches have been applied to the brains of subjects with various forms of neuronal ceroid lipofuscinosis. Conventional MR imaging and CT have been used to demonstrate the presence of cerebral atrophy in multiple forms of neuronal ceroid lipofuscinosis.\(^{15,16}\) However, atrophic grading using conventional MR imaging is only a subjective measure of disease severity. A diffuse hyperintensity of cerebral white matter has been reported in LINCL patients.\(^{2,11}\)

Postmortem studies of LINCL and juvenile neuronal ceroid lipofuscinosis patients confirm a loss of myelin and gliosis in periventricular white matter.\(^{2}\) Consistent with these findings, our patients presented with enlarged sulci and ventricles in both the supratentorial and infratentorial compartments of the brain consistent with marked atrophy. In addition, high signal intensity on T2-weighted images was observed in the periventricular white matter. A previously published CT study also showed increased subarachnoid and ventricular spaces, which were correlated with patient age.\(^ {16}\) However, CT findings were usually normal in patients younger than 10 years of age, and these findings did not correlate with onset or severity of the abnormalities, possibly reflecting the diverse forms of neuronal ceroid lipofuscinosis in this study before genetic testing.\(^ {16}\)

MR spectroscopic imaging (MRSI) has also been used to noninvasively assess brain metabolism in various neurodegenerative diseases, such as Alzheimer and Parkinson diseases.\(^ {7,17}\) \(^{1}\)H-MR spectroscopy has used single voxel techniques to determine abnormal metabolic changes in patients with LINCL within specific regions, such as the white matter of the parietal lobe.\(^ {18}\) MRSI is a sensitive though nonspecific method for assessing metabolic changes in patients with LINCL compared with normal control subjects. Multivoxel chemical shift imaging may provide further information about metabolic degradation in specific regions of the brain.\(^ {19}\)

Traditionally, DWI has been used to assess isotropic restriction of water molecules in stroke or white matter diseases of the brain, such as multiple sclerosis or ischemic leukoaraiosis.\(^ {20,21}\) DWI has rarely been used to examine neurodegenerative diseases of the brain, which primarily affect gray matter. One study examined patients with Huntington disease with DWI and found a correlation ($P = .05$) between disease stage and the mean of the whole-brain ADC histograms.\(^ {22}\) Our study showed a strong correlation between whole-brain ADC values and age along with both disease severity and duration in patients with LINCL.

This study examined the water diffusivity of the entire brain through the use of DWI. Whole-brain ADC histograms were created that included all of the voxels in the brain having signal intensities greater than a predefined threshold. This method eliminated user subjectivity required to place regions of interest on the images. The reproducibility of whole-brain ADC histograms has been confirmed as an accurate method to quantify water diffusion in a robust and objective manner.\(^ {23}\) The whole-brain histogram was fitted using dual Gaussian functions in addition to a partial volume function to characterize brain parenchyma while excluding partial volume and

*0.065 * Age (years) + 0.564] yielding $R^2 = 0.71$ ($P < .0001$).
CSF contamination. The global maximum of this function yielded an estimate of the whole-brain ADC value. As expected, compartments identified as brain parenchyma, partial volume, or CSF corresponded with those specific locations in the brain. The fitted Gaussian function with the lowest mean ADC corresponded with that of parenchyma containing both gray and white matter regions. Voxels labeled as containing partial volume fractions in the model were found to correspond with those regions at the boundary between gray and white matter and CSF. As the ADC value increased, voxels along the gray matter boundary were seen to migrate toward regions of CSF. Similarly, the voxels with high ADC were associated with the CSF compartment. Voxels having higher ADC values than CSF were also observed, most likely because of CSF flow artifacts in the region.

A significant correlation between increased whole-brain ADC values and patient age was found for all of the patients. Deviation of whole-brain ADC values from the control subjects became significant at 5 years of age, suggesting a common age of disease onset. This is consistent with the uniform age of manifestation of LINCL clinical symptoms. Because of the progressive increase of ADC values with age and the uniform age at diagnosis, it follows that the ADC values also correlate with time since diagnosis. Similarly, because severity increases with age, it would be expected that ADC values correlate with severity, which is indeed the case, though this correlation is less strong.

Changes in the ADC values were likely the result of a combination of physiologic mechanisms that primarily alter the restrictive nature of gray matter and, to a lesser degree, that of white matter. A study found that calcium-binding proteins linked to GABAergic interneurons in the cortex and cerebellum were disrupted in patients with LINCL. An additional postmortem study of 13 case subjects with LINCL found a moderate-to-severe loss of myelin and mild-to-moderate gliosis in the periventricular white matter of 10 of 13 case subjects. Increased signal intensity in the periventricular white matter on T2-weighted MR images in patients with LINCL was correlated with histology confirming atrophy. These findings implied a loss of neuronal integrity in gray matter and decreased myelination in white matter. This is consistent with the increased whole-brain ADC values found in this study showing the progressive nature of the disease.

Whole-brain ADC values correlated better with patient age than the modified CNS disability scale. Whole-brain ADC values may, thus, provide a more accurate physiologically based indicator of disease progression and severity. The objective acquisition and analysis criterion of whole-brain ADC histograms is an attractive feature of this technique. In addition, ADC measures provide a more continuous scale with which to assess severity in comparison with the discrete characterization of patient disability.

Conclusions
This study was conducted to evaluate whether quantitative data derived by DWI techniques can supplement clinical disability scale information to provide a quantitative estimate of neurodegeneration, as well as disease progression and severity. The results presented are consistent with known conventional MR imaging findings but suggest an objective and complementary technique to monitor disease progression. Increased whole-brain ADC values agree with imaging results characteristic of LINCL. Whole-brain ADC values were significantly correlated with patient age, disease severity as assessed with the modified CNS disability scale, and disease duration. DWI has been shown to detect variations in cerebral water diffusion abnormalities in patients with LINCL and may have the potential to monitor disease progression and severity in conjunction with the clinical characterization of patient disability.

References
1. Crystal RG, Sondhi D, Hackett NR, et al. Clinical protocol. Administration of a replication-deficient adenovirus-associated virus gene transfer vector expressing the human CLN2 cDNA to the brain of children with late infantile neuronal ceroid lipofuscinosis. Hum Gene Ther 2004;15:1131–54
2. Autti T, Raininko R, Santavuori P, et al. MRI of neuronal ceroid lipofuscinosis. II. Postmortem MRI and histopathological study of the brain in 16 cases of neuronal ceroid lipofuscinosis of juvenile or late infantile type. Neuropathology 1997;39:371–77
3. Sondhi D, Hackett NR, Aphrnt RL, et al. Feasibility of gene therapy for late neuronal ceroid lipofuscinosis. Arch Neurol 2001;58:1793–98
4. Steinfeld R, Heim P, von Gregory H, et al. Late infantile neuronal ceroid lipofuscinosis: quantitative description of the clinical course in patients with CLN2 mutations. Am J Med Genet 2002;112:347–54
5. Balbon D, Dyke J, Schwartz LH, et al. Imaging therapeutic response in human bone marrow using rapid whole-body MRI. NMR Biomed 2000;13:321–28
6. Bosma GP, Huizinga TW, Mooijaart SP, et al. Abnormal brain diffusivity in patients with neuropsychiatric systemic lupus erythematosus. AJNR Am J Neuroradiol 2003;24:850–54
7. Santarci K. Magnetic resonance markers for early diagnosis and progression of Alzheimer’s disease. Expert Rev Neurother 2005;5:663–70
8. Ulag AM. Monitoring brain development with quantitative diffusion tensor imaging. Develop Sleep 2002;5:286–92
9. Mukherjee P, McKinstry RC. Diffusion tensor imaging and tractography of human brain development. Neuroimag Clin 1996;6:18–43
10. Lauterlaw DH, Fleischer KW, Barr AH. Partial-volume Bayesian classification of material mixtures in MR volume data using voxel histograms. IEEE Trans Med Imag 1998;17:74–86
11. Chun T, Filippi CG, Zimmerman RD, et al. Diffusion changes in the aging human brain. AJNR Am J Neuroradiol 2000;21:1078–83
12. Hedges S, Shah P. Comparison of mode estimation methods and application in molecular cell analysis. BMC Bioinformatics 2003;4:43
13. Narolci N, Verga ML, Binelli S, et al. Neuronal ceroid-lipofuscinosis: a clinical and morphological study of 19 patients. Am J Med Genet 1995;57:137–41
14. Santavuori P, Vanhanen SL, Autti T. Clinical and neuroradiological diagnostic aspects of neuronal ceroid lipofuscinosis disorders. Eur J Paediatr Neurol 2001;5:157–61
15. Vanhanen SL, Puranen J, Autti T, et al. Neuroradiological findings (MRS, MRI, SPECT) in infantile neuronal ceroid lipofuscinosis (infantile CLN1) at different stages of the disease. Neuropediatrics 2004;35:27–31
16. Logstein I, Schwendemann G, Kuhn D, et al. Neuronal ceroid-lipofuscinosis: CFT findings in fourteen patients. Acta Paediatr Scand 1981;70:857–60
17. Seppi K, Schocke MF. An update on conventional and advanced magnetic resonance imaging techniques in the differential diagnosis of neurodegenerative Parkinsonism. Curr Opin Neurol 2005;18:570–75
18. Sett D, Grodd W, Schwab A, et al. MR imaging and localized proton MR spectroscopy in late infantile neuronal ceroid lipofuscinosis. AJNR Am J Neuroradiol 1998;19:1373–77
19. Shungu DC, Worgall S, Mao X, et al. Spectral characteristics of late infantile neuronal ceroid lipofuscinosis (“Batten disease”) investigated in vivo by 1H magnetic resonance spectroscopic imaging at 3.0 T. Proc Int Soc Magn Res Med 2005;15:1268
20. Mascacchi M, Moretti M, Della Nave R, et al. Longitudinal evaluation of leukoraiosis with whole brain ADC histograms. Neurology 2002;59:938–40
21. Wilson M, Morgan PS, Lin X, et al. Quantitative diffusion weighted magnetic resonance imaging, cerebral atrophy, and disability in multiple sclerosis. J Neurol Neurosurg Psychiatry 2001;70:218–22
22. Mascacchi M, Lolli F, Della Nave R, et al. Huntington disease: volumetric, diffusion-weighted, and magnetization transfer MR imaging of brain. Radiology 2004;232:867–73
23. Steens SC, Admiraal-Behloul F, Schaap JA, et al. Reproducibility of brain ADC histograms. Eur Radiol 2004;14:423–30
24. Hachiya Y, Hayashi M, Kumada S, et al. Mechanisms of neurodegeneration in neuronal ceroid-lipofuscinosis. Acta Neuropathol (Berl) 2006;111:168–77