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Original Article

Hippocampal Volumes in Juvenile Neuronal Ceroid Lipofuscinosis: A Longitudinal Magnetic Resonance Imaging Study

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ABSTRACT

BACKGROUND: Juvenile neuronal ceroid lipofuscinosis is an inherited, autosomal recessive, progressive, neurodegenerative disorder of childhood. It belongs to the lysosomal storage diseases, which manifest with loss of vision, seizures, and loss of cognitive and motor functions, and lead to premature death. Imaging studies have shown cerebral and cerebellar atrophy, yet no previous studies evaluating particularly hippocampal atrophy have been published. This study evaluates the hippocampal volumes in adolescent juvenile neuronal ceroid lipofuscinosis patients in a controlled 5-year follow-up magnetic resonance imaging study. **METHODS:** Hippocampal volumes of eight patients (three female, five male) and 10 healthy age- and sex-matched control subjects were measured from two repeated magnetic resonance imaging examinations. Three male patients did not have controls and were excluded from the statistics. In the patient group, the first examination was performed at the mean age of 12.2 years and the second examination at the mean age of 17.3 years. In the control group, the mean ages at the time of examinations were 12.5 years and 19.3 years. **RESULTS:** Progressive hippocampal atrophy was found in the patient group. The mean total hippocampal volume decreased by 0.85 cm^3 during the 5-year follow-up in the patient group, which corresponds to a 3.3% annual rate of volume loss. The whole brain volume decreased by 2.9% per year. The observed annual rate of hippocampal atrophy also exceeded the previously reported 2.4% annual loss of total gray matter volume in juvenile neuronal ceroid lipofuscinosis patients. **CONCLUSIONS:** These data suggest that progressive hippocampal atrophy is one of the characteristic features of brain atrophy in juvenile neuronal ceroid lipofuscinosis in adolescence.

Keywords: juvenile neuronal ceroid lipofuscinosis, JNCL, NCL, CLN3, Batten disease, lysosomal storage disease, hippocampus, MRI volumetry

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Introduction

The neuronal ceroid lipofuscinoses (NCLs) are recessively inherited, lysosomal storage diseases and constitute the most common group of progressive encephalopathies of childhood.[1] Juvenile neuronal ceroid lipofuscinosis (JNCL, CLN3, Batten disease, Spielmeyer-Vogt-Sjögren disease) is the most common form of NCLs, with an incidence of 2.0 to 7.0 per 100,000 births in Scandinavia.[2] In central Europe, the reported incidence varies from 0.2 to 1.5 per 100,000 births.[3,4] In Canada, the incidence is estimated to be 0.6 per 100,000 births.[5] The disease is caused by a mutation in *CLN3* gene. The absence of normally functioning CLN3 protein seems to affect numerous cellular functions and there is accumulation of autofluorescent storage material in most tissues.[1,6-9] Children with JNCL are born healthy. Around the age of 5 years, visual impairment is noticed. In early school

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years, learning difficulties become evident and, around the age of 10 years, the first epileptic seizures occur.[10] Short-term memory is deficient already at an early age in JNCL and digit memory span is one of the first functions to be impaired.[11] The disease leads to premature death between the ages of 16 and 35 years.[12]

Magnetic resonance imaging (MRI) is a noninvasive method that is able to reveal pathological alterations in brain tissue. In previous MRI studies involving JNCL patients, a mild to moderate cerebral and cerebellar atrophy has been found as well as a decreased T2 signal intensity of the thalamus.[12-17] Decreased gray matter volume has also been found in a study using voxel-based morphometry.[18]

The hippocampus is a paired cortical gray matter structure in the medial surface of the temporal lobe. It belongs to the limbic system and is involved in memory storage, retrieval, and regulation of emotion. Furthermore, the hippocampus is connected with the thalamus via the mammillothalamic tract. Hippocampal atrophy is a common feature in many neurodegenerative diseases and is also associated with epilepsy.[19-24] Bearing in mind that progressive memory decline and epilepsy are typical symptoms of JNCL, it is interesting to determine hippocampal volumes in this disease and evaluate hippocampal atrophy in comparison with general brain atrophy.

MRI-based measurement of hippocampal volume is an accepted and frequently used method of investigation.[25,26] It has revealed alterations in hippocampal volume in a variety of neurological and psychiatric disorders. In addition to the manual measurement method used in this study, automated methods for the measurement of hippocampal volumes have been developed (e.g., Free Surfer, IBASPM).

Methods and Materials

We investigated eight JNCL patients (three girls, five boys) and 10 healthy age- and sex-matched control subjects (six girls, four boys). Three male patients did not have controls and were excluded from the statistics. Their results are presented separately. Therefore, we had five patients (three girls and two boys) with double controls in the study group included in the statistics. The JNCL diagnoses of four of these patients were confirmed by DNA analysis. In one patient, the diagnosis was based on vacuolated lymphocytes and ophthalmologic findings. All patients were blind and had mental decline. At the onset of the study, all patients received antiepileptic medication, yet three of five had not had any seizures. The seizure frequency and antiepileptic medication at the time of the first examination is shown in Table 1. One patient had her first seizure during the follow-up; otherwise, the seizure frequency among patients remained stable. On the whole, none of the patients developed severe epilepsy. The healthy control subjects were volunteers recruited for this study. They had no history of neurological or psychiatric symptoms, took no medication, and had a normal school history.

Each patient and control had two MRI examinations performed. These patients had previously been included in a voxel-based morphometry study[18] and a volumetric study of the brain.[17] We reevaluated the three-dimensional MRI scans of such patients whose image quality was considered sufficient for hippocampal volumetry. In the patient group, the first examination was performed at the mean age of 12.2 years (standard deviation [SD] 1.0) and the second examination at the mean age of 17.3 years (SD 1.1). The mean time period between the examinations was 5.2 years (SD 0.5). In the control group, the mean ages at the time of examinations were 12.5 years (SD 1.1) and 19.3 years (SD 1.1), and the mean time period between the examinations was 6.8 years (SD 0.7). The results of the measurements are shown in Table 2.

Hippocampal volumes of the three male JNCL patients, who did not have age- and sex-matched controls, were also measured. Their ages at the onset of the study were 16.0, 19.0, and 31.5 years. These patients were also examined twice and the time period between the examinations was 5.3 to 5.4 years. The oldest and the youngest were heterozygous for the major mutation. The patients' epileptic seizure frequency and medication they took is shown at the end of Table 1. Their seizure frequency also remained stable during follow-up. The results of the hippocampal volume measurements of these three patients were excluded from the statistics and their values are shown in Table 3.

MRI scans were acquired on a 1.5 T scanner (Siemens Magnetom Vision). We used a three-dimensional magnetization prepared rapid acquisition gradient echo (3D-MPRAGE) sequence (repetition time = 9.7 msec, echo time = 4 msec, flip angle 10 or 12, no interslice gap), matrix size 256 × 256 × 170 pixels and isotropic voxel size of 1.0 × 1.0 × 1.0 mm. The images were saved as collections of sagittal slices but for the analysis three-dimensional volumes were reconstructed. Regions of interests (ROI) defining right and left hippocampi were drawn using an in-house–developed medical image processing software. The software enabled working on sagittal, axial, and coronal views. The drawn ROIs could be visualized transparently superimposed on the original MRI data using orthogonal views with freely chosen slice directions. Total hippocampal volume (right + left = total) was used in the statistics.

Although MRI-based hippocampal volumetry is an accepted technique, there is variation among protocols in image acquisition, image processing, and anatomical guidelines.[25-28] The cornu ammonis, gyrus dentatus, presubiculum, and subiculum proper were included in the measurements. The anatomical tracing was first performed in sagittal views. An ROI was drawn to define right and left hippocampi in all the sagittal slices where they were visible. The drawings were then projected onto the coronal views and the anatomical boundaries were checked. This was done separately for every MRI examination. Tracing began on the most lateral slice on which the hippocampus was first visible in the ventricular temporal horn. The medial border was determined by the slice on which the hippocampus was clearly distinct from the amygdala. Anteriorly, the hippocampus was separated from the amygdala by a thin line of white matter that is visible between the structures. Posteriorly, the fornices were not included in the drawings. The inferior border was determined by a line of white matter separating the hippocampus from the parahippocampal gyrus and superior border by a line separating the hippocampus from the lateral ventricles. Examples of ROI drawings of are illustrated in Figure 1. The measured hippocampal volumes were normalized with the total intracranial volumes

TABLE 1.
Patients

Patients	Genotype	Age at Onset	Medication	Seizure Frequency
Patient 1	Homozygous	10.8	Lamotrigine	0
Patient 2	Homozygous	11.8	Lamotrigine	0
Patient 3	Homozygous	11.9	Lamotrigine	0
Patient 4	Homozygous	12.6	Lamotrigine	4/yr
Patient 5	Unknown	13.6	Lamotrigine, clonazepam	3/yr
Patient 6	Compound heterozygous	16.0	Lamotrigine, clonazepam	2/yr
Patient 7	Homozygous	19.0	Valproate	1/yr
Patient 8	Compound heterozygous	31.5	Lamotrigine	0

Patients 6-8 are excluded from statistics (no matched control subjects).