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Abnormalities of hippocampal signal intensity in patients with familial mesial temporal lobe epilepsy

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Abstract

Mesial temporal lobe epilepsy (MTLE) is associated with hippocampal atrophy and hippocampal signal abnormalities. In our series of familial MTLE (FMTLE), we found a high proportion of hippocampal abnormalities. To quantify signal abnormalities in patients with FMTLE we studied 152 individuals (46 of them asymptomatic) with FMTLE. We used NIH-Image® for volumetry and signal quantification in coronal T1 inversion recovery and T2 for all cross-sections of the hippocampus. Values diverging by 2 or more SD from the control mean were considered abnormal. T2 hippocampal signal abnormalities were found in 52% of all individuals: 54% of affected subjects and 48% of asymptomatic subjects. T1 hippocampal signal changes were found in 34% of all individuals: 42.5% of affected subjects and 15% of asymptomatic subjects. Analysis of the hippocampal head (first three slices) revealed T2 abnormalities in 73% of all individuals (74% of affected subjects and 72% of asymptomatic subjects) and T1 abnormalities in 59% (67% of affected subjects and 41% of asymptomatic subjects). Affected individuals had smaller volumes than controls ($P < 0.0001$). There was no difference in hippocampal volumes between asymptomatic subjects and controls, although 39% of asymptomatic patients had hippocampal atrophy. Patients with an abnormal hippocampal signal (133 individuals) had smaller ipsilateral volume, but no linear correlation could be determined. Hippocampal signal abnormalities in FMTLE were more frequently found in the hippocampal head in both affected and asymptomatic family members, including those with normal volumes. These results indicate that subtle abnormalities leading to an abnormal hippocampal signal in FMTLE are not necessarily related to seizures and may be determined by genetic factors.

Key words
- Hippocampal signal abnormalities
- Familial mesial temporal lobe epilepsy
- Hippocampal volumetry
- Magnetic resonance imaging

Introduction

Hippocampal atrophy is a frequent magnetic resonance imaging (MRI) finding in mesial temporal lobe epilepsy (MTLE). Hippocampal atrophy is correlated with the pathological feature of mesial temporal sclerosis (MTS) (1-8), which is characterized by selective neuronal loss mainly in the hippocampus, but also in the amygdala and parahippocampus (2).

Although highly correlated to refractory

seizures, hippocampal atrophy has also been described in patients with benign MTLE. In familial MTLE (FMTLE), hippocampal atrophy determined by volumetry was associated with abnormal internal structure and an increased T2 signal (by visual analysis) in individuals with a single partial seizure or seizure remission, as well as in 34% of asymptomatic family members (9-12).

The finding of clear-cut hippocampal atrophy in these familial patients indicates the presence of genetic factors predisposing to hippocampal damage. The genetic basis of FMTLE may determine a wide range of clinical and MRI abnormalities, from absolutely normal MRI in asymptomatic individuals to severe hippocampal atrophy with a hyperintense T2 signal in refractory patients.

The use of quantitative MRI techniques provides objective data and can be a useful tool for the understanding of the underlying mechanisms involved in hippocampal damage (6,7,13-16). It has been shown that reduced hippocampal volumes are associated with neuronal loss (7), whereas a hyperintense T2 signal is more likely to reflect gliosis in hippocampal formation (16).

Studies using T2 signal quantification (relaxometry) have shown a high sensitivity and specificity for hippocampal signal abnormalities (7,13-16). However, they used specific T2 acquisitions, with many echo times, that may not be available in some MRI systems. In addition, relaxometry is focused on a single hippocampal section, limiting its use in a three-dimensional complex structure such as the hippocampal formation.

The objectives of the present study were to quantify T1 and T2 hippocampal signals in patients with FMTLE using standard one-echo MRI, and to correlate these findings with clinical presentation and hippocampal volumes.

Patients and Methods

We studied 152 individuals (106 affected and 46 asymptomatic subjects) from 36 unrelated families with FMTLE. Of these individuals, 89 were women. All families had at least two first- or second-degree relatives with a diagnosis of MTLE by clinical and electroencephalogram data. None of these families had any affected individual with suspected extra-temporal epilepsy, or semiology compatible with neocortical temporal lobe epilepsy. Asymptomatic family members were all first-degree relatives of patients with MTLE.

All patients and asymptomatic family members signed an informed consent for this study, which was approved by the Ethics Committee of our hospital.

A detailed clinical description and pedigrees of these families (10), as well as visual assessment of hippocampal signal abnormalities (11,12), have been published.

The control group consisted of 40 healthy adult volunteers (19 women). All MRIs were obtained with a 2 Tesla scanner (Elscint Prestige®, Haifa, Israel), with T1- and T2-weighted acquisitions on three orthogonal planes. For volumetry and hippocampal signal quantification we used coronal (3 mm) T1 inversion recovery (IR) and T2 images, with slices oriented perpendicularly to the long axis of the hippocampus to optimize the evaluation of mesial temporal structures.

MRI acquisition parameters were: T2-weighted "fast spin echo", 4-mm thick, flip angle = 120º, TR = 4800, TE = 129, matrix 252 x 320, FOV = 18 x 18 cm, and T1-weighted IR, 3-mm thick, flip angle = 200º, TR = 2800, TE = 14, inversion time = 840, matrix 130 x 256, FOV = 16 x 18 cm.

Analyses were performed on a Power MacIntosh G4 computer using the NIH-Image® program (developed at the National Institutes of Health, Bethesda, MD, USA, and available on the Internet at http://www.rsb.info.nih.gov/nih-image). NIH-Image provides the average gray value within the selected regions of interest and this value is the sum of the gray values of all pixels in

the selection divided by the number of pixels. In addition, it is possible to determine volumes of structures using the area of the regions of interest.

Hippocampal volumetry was performed according to a standard protocol (13,15) using coronal T1-IR slices. We calculated hippocampal volumes corrected by the variation in total intracranial volume, and the hippocampal asymmetry index for each patient, and transformed these data into Z-scores (number of standard deviations (SD) from the mean for the control group). Z scores below -2 SD were considered abnormal.

We used the same software for quantification of the hippocampal signal in both T1 and T2 coronal slices. The inner boundaries of the hippocampal formation were manually delineated throughout the extension of the structure. In addition to the determination of the hippocampal signal from the whole hippocampal formation, we calculated the hippocampal head signal (Hip-head-signal), determined as the mean value for the first three slices of the hippocampal formation. Values were corrected by the signal measured on the midline portion of the pons, and transformed into Z-scores. This technique was validated in a group of MTLE patients submitted to surgical treatment (17).

An abnormal T1 hippocampal signal (hypointense) was determined for values below 2 SD from the mean of the control group and an abnormal T2 hippocampal signal (hyperintense) for values above 2 SD from the mean of the control group.

Statistical analyses were performed using the SYSTAT9® software. We performed analysis of variance (ANOVA) to assess differences in continuous variables among groups, with *post hoc* pairwise comparisons (Tukey test). The chi-square test was used to determined the differences in the frequency distribution of abnormalities between groups. Pearson's simple correlation was calculated between hippocampal volumes and hippocampal signal.

Results

Frequency distribution

A total of 94 (62%) individuals with hippocampal signal abnormalities were detected: 52 with unilateral (36 right) and 42 with bilateral abnormalities.

T2 hippocampal signal abnormalities were found in 79 (52%) subjects: 54% of them affected and 48% asymptomatic. T1 hippocampal signal changes were found in 52 (34%) individuals, 42.5% of them affected and 15% asymptomatic. An abnormal T2 hippocampal signal was equally frequent in affected and asymptomatic individuals, while an abnormal T1 hippocampal signal was significantly more frequent in affected individuals (P = 0.002, chi-square test) (Table 1).

As expected, signal analysis limited to the hippocampal head revealed more frequent abnormalities. There were 130 (85%) individuals with either a T1 or T2 abnormal Hip-head-signal, unilateral in 62 (50 right) and bilateral in 68. We found T2 Hip-head-signal changes in 111 (73%) individuals (74% of them affected and 72% asymptomatic) and T1 Hip-head-signal abnormalities in 90

Table 1. Frequency of abnormal signal and hippocampal atrophy in affected and asymptomatic individual members of families with familial mesial temporal lobe epilepsy.

	Affected (N = 106)	Asymptomatic (N = 46)	Total (N = 152)
T2 signal*	57 (54%)	22 (48%)	79 (52%)
T1 signal*	45 (43%)	7 (15%)	52 (34%)
Either T2 or T1 signal*	73 (69%)	21 (46%)	94 (62%)
T2 hippocampal head signal**	78 (74%)	33 (72%)	111 (73%)
T1 hippocampal head signal**	71 (67%)	19 (41%)	90 (59%)
Either T2 or T1 hippocampal head signal**	97 (92%)	33 (72%)	130 (85%)
Hippocampal atrophy	72 (68%)	18 (39%)	90 (59%)

An abnormal signal is defined as values above (T2) or below (T1) ± 2 SD from the mean value of the controls. Hippocampal atrophy is defined as values 2 SD below the mean value of the controls (volumes corrected for the variation of total brain volume). *Average signal intensity of the entire hippocampus. **Average signal intensity of the hippocampal head only.

(59%) individuals (67% of them affected and 41% asymptomatic). Again, T2 Hip-head-signal abnormalities were equally frequent in the affected and asymptomatic groups, but a T1 Hip-head-signal was more frequent in the group of affected individuals (P = 0.003; Table 1).

Hippocampal atrophy was found in 90 (59%) individuals, bilateral in 50 of them and unilateral in 40 (24 left). These abnormalities were found in 72/106 (68%) affected and 18/46 (39%) asymptomatic individuals belonging to MTLE families (Table 1).

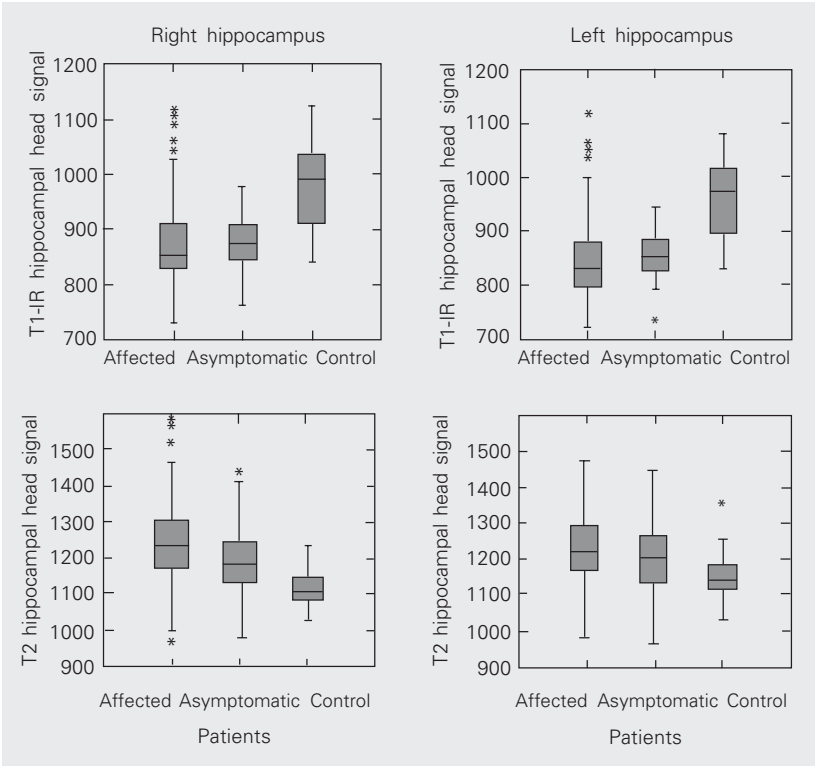

Figure 1. Box-whisker plot showing that affected (N = 106) and asymptomatic (N = 46) family members had significantly lower T1 and T2 signals compared to controls (N = 40). In these box plots, the center vertical line inside the box indicates the median value of the sample. The length of each box shows the range within which the central 50% of the values fall, with the box edges (called hinges) at the first and third quartiles (i.e., 25 and 75% of the sample, respectively). The straight vertical lines (whiskers) extend from the smallest (bottom) to the largest (top) observation. Asterisks represent outliers. The Y axis shows the values of each variable (i.e., T1, T2 signal for the right and left hippocampus). The groups are identified on the X axis. ANOVA showed a significant difference for T1 and T2 hippocampal signal values from the left and right sides among groups (P < 0.0000001). *Post hoc* pairwise comparisons by the Tukey test showed significant differences between controls and each of the other two groups (P values varying from 0.0003 to 0.000002 for each paired comparison), except for the comparison of left T2 hippocampal signal, where there was no significant difference between unaffected subjects and controls (P = 0.057). T1-IR = T1 inversion recovery.

Hippocampal volumes were smaller in the patient group compared to control (P < 0.0001, ANOVA with pairwise Tukey comparisons), but no group differences were found between asymptomatic family members and controls. Patients with an abnormal T1 or T2 hippocampal signal had smaller ipsilateral hippocampal volumes, but no significant linear correlation between these two variables could be determined.

An abnormal T2 or T1 hippocampal signal was lateralized in 24/50 (48%) individuals with bilateral hippocampal atrophy, and identified abnormalities in 34/62 (55%) individuals with normal hippocampal volumes. Unilateral discordant hippocampal signal abnormalities were identified in only 6 individuals (3 affected and 3 asymptomatic subjects) with unilateral hippocampal atrophy.

Comparisons of mean values among groups

ANOVA showed a significant difference in T1 and T2 hippocampal signal values from the left and right sides among groups (P < 0.0000001). *Post hoc* pairwise comparisons by the Tukey test showed a significant difference between the controls and each of the other two groups (P values varying from 0.0003 to 0.000002 for each paired comparison), except for the comparison of left T2 hippocampal signal, which showed no significant difference between unaffected subjects and controls (P = 0.057) (Figure 1).

ANOVA showed a significant difference among groups for left and right hippocampal volumes (P < 0.0001, Figure 2). *Post hoc* pairwise comparisons by the Tukey test showed a significant difference between affected subjects and the other two groups (P = 0.00001 and 0.000006 for the right and left hippocampus of unaffected versus affected subjects; P = 0.005 and 0.0004 for right and left hippocampus of controls versus affected

subjects), but no difference between asymptomatic subjects and controls (P = 0.98 for the right hippocampus and P = 0.96 for the left hippocampus).

Relationships between hippocampal signal and hippocampal volume abnormalities

Patients with an abnormal T2 or T1 hippocampal signal had significantly smaller ipsilateral hippocampal volumes compared to patients with a normal T2 and T1 hippocampal signal (ANOVA, P = 0.0002 for the left side and P < 0.0001 for the right side), but there was no significant correlation between hippocampal volumes and ipsilateral T1 (P = 0.149, r = 0.11 for the right hippocampus/P = 0.59, r = 0.04 for the left hippocampus) or T2 signal (P = 0.09, r = 0.13 for the right hippocampus, P = 0.14, r = 0.11 for the left hippocampus).

Discussion

Several studies have reported T2 signal abnormalities, suggesting that the signal correlates with gliosis and may not be directly related to the degree of neuronal loss (6,7,14,16). However, all of these studies included only patients with refractory epilepsy who underwent surgical treatment. We report here the first observations of T1 and T2 signal changes in individuals who were not surgical candidates, including asymptomatic first-degree relatives of patients with FMTLE.

The recent recognition of FMTLE has provided evidence for a genetic factor involved in the development of hippocampal abnormalities in these patients (9,10,16). Visually detectable abnormalities in hippocampal signal have been reported for most of FMTLE-affected individuals (10,12) and for asymptomatic family members (11). Hippocampal signal quantification, however, can be helpful to determine first whether there really are signal abnormalities in these individuals, and second, if there is a correlation of the affected status and hippocampal volumes.

In the present study, we observed frequent hippocampal signal abnormalities in affected and asymptomatic individuals belonging to families with MTLE. Signal changes were more severe and often restricted to the anterior portion of the hippocampus (head); therefore, averaging signal values from the entire hippocampus may reduce sensitivity. In addition, measuring signal only in the anterior portion of the hippocampus is less time consuming.

T2 signal changes were more frequent and more severe than T1 signal changes among affected and unaffected individuals; however, T1 signal changes appeared to discriminate better between affected and unaffected individuals. This may indicate that significant T1 signal changes occur only in more advanced hippocampal pathology, which is consistent with our observation in patients with MTLE undergoing pre-surgical investigation (17).

T2 signal abnormalities were not identi-

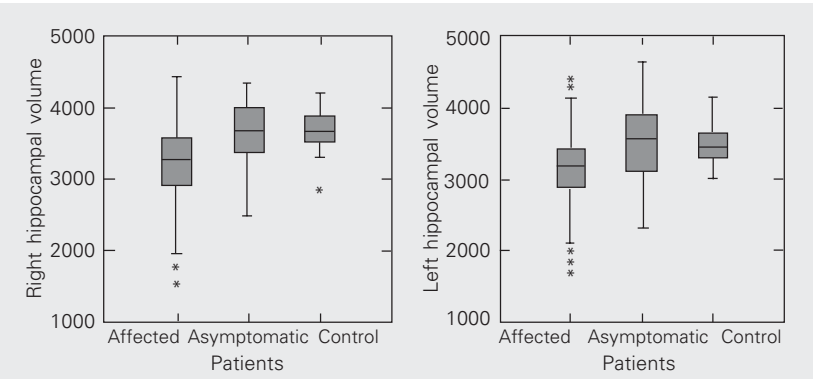

Figure 2. Box-whisker plot summarizing the results of right and left hippocampal volumes for the three groups (affected, asymptomatic and control). ANOVA showed a significant difference among groups for left and right hippocampal volumes (P < 0.0001). *Post hoc* pairwise comparisons by the Tukey test showed significant differences between affected subjects (N = 106) and each of the other two groups (P = 0.00001 and P = 0.000006 for comparisons of right and left hippocampus of unaffected (N = 46) versus affected subjects; P = 0.005 and P = 0.0004 for comparisons of right and left hippocampus of controls (N = 40) versus affected subjects); but no difference between asymptomatic subjects and controls (P = 0.98 for right hippocampus and P = 0.96 for left hippocampus). Asterisks represent outliers.

fied in all affected subjects; in addition, they were present in 48% of unaffected individuals. These observations may indicate that some asymptomatic individuals are carriers of the unidentified gene mutation. Alternatively, one may argue that there is no direct association between seizures and an abnormal T2 signal. Since the causative gene in FMTLE has not yet been identified, we can only speculate that the relationship between this putative gene mutation and the occurrence of seizures or MRI abnormalities may be more complex than expected. Furthermore, we observed that 55% of patients with normal hippocampal volumes had an abnormal hippocampal signal. Taken together, these data may support the existence of less severe MTS or pre-existing abnormalities, that appear to be inherited and that may predispose to the development of classical MTS in these individuals (10-12). At the present time, it is unclear which specific roles genetic and environmental factors play in the development of MTS in FMTLE. Further investigations will be required in order to access the pathological substrate that leads to the *in vivo* T1 and T2 signal changes in FMTLE.

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