Neuropathologic and Clinical Features of Human Medial Temporal Lobe Epilepsy

Eun-Kee Bae, MD, PhD<sup>a,b,c</sup>; Keun-Hwa Jung, MD, PhD<sup>a,b,c,e</sup>; Kon Chu, MD, PhD<sup>a,b,c,e</sup>; Soon-Tae Lee, MD, PhD<sup>b</sup>; Jin-Hee Kim, MD, PhD<sup>a</sup>; Kyung-II Park, MD, PhD<sup>d</sup>; Manho Kim, MD, PhD<sup>a,b</sup>; Chun-Ki Chung, MD, PhD<sup>c,e</sup>; Sang Kun Lee, MD, PhD<sup>a,b,c</sup>; Jae-Kyu Roh, MD, PhD<sup>a,b</sup>

<sup>a</sup>Stroke & Stem Cell Laboratory in Clinical Research Institute, Stem Cell Research Center, Department of Neurology, <br><sup>b</sup>Program in Neuroscience, Neuroscience Research Institute of SNUMRC, Seoul National University, Seoul, Korea <br><sup>c</sup>Comprehensive Epilepsy Center, Seoul National University Hospital, Seoul, Korea <br><sup>d</sup>Department of Neurology, Seoul Paik Hospital, Inje University College of Medicine, Seoul, Korea <br><sup>e</sup>Department of Neurosurgery, Seoul National University Hospital, Seoul, Korea

Received November 12, 2009 <br>Revised March 20, 2010 <br>Accepted March 23, 2010

Correspondences<br>Sang Kun Lee, MD, PhD<br>Department of Neurology, Seoul National University Hospital, 101 Daehak-ro, Jongno-gu, Seoul 110-744, Korea<br>Tel +82-2-2072-2923<br>Fax +82-2-3672-4949<br>E-mail sangunlee@dreamwiz.com<br>Jae-Kyu Roh, MD, PhD<br>Department of Neurology, Seoul National University Hospital, 101 Daehak-ro, Jongno-gu, Seoul 110-744, Korea<br>Tel +82-2-2072-3265<br>Fax +82-2-3672-4949<br>E-mail rohjk@snu.ac.kr

*The authors contributed equally to this study.

Background and Purpose There is recent evidence of various types of morphological changes in the hippocampus of a rodent model of medial temporal lobe epilepsy (mTLE). However, little is known about such changes in humans. We examined the histological changes [i.e., neuronal loss, cell genesis, and granule cell dispersion (GCD)] in surgical hippocampal specimens taken from patients with mTLE.

Methods Nissl staining, and nestin and Prox1 immunohistochemistry were performed on human hippocampal specimens obtained from patients with medically intractable mTLE, thus allowing the analysis of neuronal loss, cell genesis, and GCD, respectively. We also assessed the correlations between clinical parameters and the histopathologic findings.

Results The degree of cell genesis in the granule cell layer was significantly correlated with the severity of GCD, history of childhood febrile seizures, and frequent generalized seizures. Cell genesis was not correlated with cell death, age at seizure onset, duration of epilepsy, or the mean frequency of all seizures.

Conclusions Our results indicate that cell genesis in the dentate gyrus of patients with mTLE is associated with GCD and is influenced by the presence of febrile seizures during childhood and the frequency of episodes of generalized seizures.

Key Words epilepsy, temporal lobe, dentate gyrus, hippocampus, pathology.

Introduction

Hippocampal sclerosis (HS) is the most common lesional abnormality identified in patients with temporal lobe epilepsy (TLE). HS is characterized by severe loss of the principal neurons in areas CA1 and CA3 of the hippocampus, and is frequently associated with widening of the granule cell layer of the dentate gyrus, termed granule cell dispersion (GCD), which is observed in about 40-50% of surgical temporal lobe specimens. Despite their importance in TLE, the pathogenic mechanisms underlying this distinctive hippocampal pathology have not yet been identified. Whether HS represents the cause or the consequence of chronic seizure activity and pharmacoresistant TLE also remains to be established.

The clinical significance of the morphological changes observed in the hippocampi of patients with medial TLE (mTLE) is ambiguous, in spite of the many relevant studies that have been conducted over the last 50 years. Although there have been many suggestions of a positive correlation between the severity of hippocampal neural loss and clinical parameters that indicate a greater seizure burden [e.g., younger age of onset, longer duration of epilepsy, greater number of generalized sei-
juries, and presence of status epilepticus (SE) and initial precipi-
tating injuries (IPI), there is currently no reliable evidence of
such a relationship.8,13

In addition to the relationship between clinical and patho-
logical parameters, there is no established evidence of a rela-
tionship between the pathological parameters themselves. For
example, the development of GCD in the hippocampus is th-
ought to be affected by excessive neurogenesis as a result of
seizures in rodent models of TLE.14 However, there are insuffi-
cient and conflicting data about the relationship between GCD
and cell proliferation in the human hippocampus.15,16

In the study presented here, we examined the histological ch-
anges associated with TLE in terms of neuronal loss, cell gen-
esis, and GCD in surgical hippocampal specimens taken from
patients with pharmacoresistant mTLE and HS. We also exam-
ined both the clinicopathologic relationship and the interrela-
tionship between these pathologic changes to better under-
stand the clinical significance and pathologic mechanisms
underlying the histological changes in mTLE.

Methods

Subjects

Cases were selected from the pathology archives at the De-
partments of Neurology and Neurosurgery, Seoul National
University Hospital, Seoul, Korea. The research was approved
by the Hospital Committee on Human Ethics. The hippocam-
pal specimens were obtained from 26 patients who had under-
gone temporal lobectomy between 2000 and 2006, including
hippocampal resection, for the treatment of medically intracta-
ble mTLE with HS. Medically intractable patients were defined
as those whose seizures were poorly controlled with two or
more anticonvulsant drugs prescribed by an epileptologist,
including at least one of the following: phenytoin, carbamaze-
pine, and valproic acid.17 All patients went through a compre-
hensive clinical, electrophysiological, neuropsychological, and
imaging evaluation before the epilepsy surgery.

Clinical data collection

Clinical data were obtained for each patient by reviewing all
records available in the electronic medical records at the hos-
pital. These data included the patient’s sex, age at epilepsy on-
set, age at surgery, duration of epilepsy, average frequency of
preoperative complex partial and generalized seizures, histo-
ry of childhood febrile seizure (FS) or other significant IPI,18
age at FS or IPI, history of SE, and surgical outcomes.

Tissue preparation and immunohistochemistry

The brain specimens were cryopreserved as tissue blocks for
cryostat sectioning at a thickness of 7 μm. Coronal sections were
taken from the right or left hippocampus, and every seventh se-
cision of the hippocampus (6 sections per specimen) was sub-
jected to semiquantitative immunohistochemical analysis. All
hippocampal specimens were processed identically to mini-
mize the effect of differential shrinkage on neuronal density
measurements. Histological evaluations were performed as de-
scribed previously.19-21 Paraffin-embedded sections from se-
lected HS patients were dewaxed, rehydrated through graded
alcohols, and taken to water. Sections were microwaved for
15 minutes in 0.05 M EDTA (pH 7.5) and then allowed to cool
for 20 minutes.

Sections were stained using the Nissl method and labeled
with antinestin (1:100, Chemicon, Temecula, CA, USA) and
anti-Prox1 antibodies (1:60, Abcam, Cambridge, UK). Nestin,
which is a protein of the intermediate filament family, is typi-
cal of undifferentiated neural stem and progenitor cells.22
Prox1 is expressed in postmitotic dentate granule cells, is spe-
cific for this cell type in the adult rat dentate gyrus,23-25 and is
used to identify ectopic granule cells following pilocarpine-
induced SE.26,27 Biotinylated goat antimouse IgG (ABC, Sig-
ma, Poole, UK) was used as the secondary antibody. The bi-
otin signal was detected using 3,3’-diaminobenzidine (brown)
and Vector VIP (purple). Prox1 expression was detected by
nickel-enhanced diaminobenzidine peroxidase immunohisto-
chemistry, as described previously.26,27 We also examined the
extrahippocampal pathological findings. The surgically ex-
cised temporal lobe specimens were fixed in formaldehyde
after extirpation and then stained with hematoxylin and eosin.

Grading system

Histological analysis was performed in the predefined areas
of consecutive coronal blocks of 6 serial sections. The cells of
interest were evaluated in the CA3 area for hippocampal neu-
ronal damage, the hilus for nestin immunoreactivities, and
the granule cell layer for GCD. The region of interest was set to
include the proximal, middle, and distal subfields of area CA3
stratum pyramidale (magnification ×400), the whole hilar
area (magnification ×400), and the superior and inferior por-
tions of the granule cell layer (magnification ×600). It is not
possible to perform unbiased stereology in human surgical
tissue samples where the entire region of interest is not pres-
ent,28 and so we adopted the semiquantitative scoring system of
histopathological results as follows. Neuronal loss was mea-
sured on a scale of 0-3, where grades 0, 1, 2, and 3 correspond-
ed to 0%, 20%, 20-50%, and >50% of the neurons lost in the
particular subfield, respectively. The mean and SD values of
the score were calculated from the scored sections.28 Cell gen-
esis was measured on a scale of 1-4, where grade 1 was “few
or no nestin-positive cells present (<3 cells/high-power field,
HPF)”, grade 2 was “a few nestin-positive cells present (3-10

Neuropathologic Features of TLE
cells/HPF”), grade 3 was “a moderate number of nestin-positive cells present (10-100 cells/HPF),” and grade 4 was “a high number of nestin-positive cells present (>100 cells/HPF).” The mean and SD values were calculated from the scored sections for each area separately (modified from Pirttilä et al.28). GCD was also measured on a scale of 1-4, where grade 1 describes epileptic qualities and a nondispersed appearance, grade 2 describes granule cell bodies dispersed into the molecular layer forming an irregular outer border, grade 3 describes double-layer granule cell bodies organized into two layers, and grade 4 describes predominantly granule cell death (Fig. 1).3,28

Statistical analysis
The data are presented as mean±SD values. The Pearson correlation coefficient was determined to assess the associations between variables. Two groups were compared by chi-square analysis with arbitrary dichotomization, using SPSS for windows (version 15.0; SPSS Inc, Chicago, IL, USA). A value of \( p<0.05 \) was considered significant.

Results
Clinical characteristics of the patients
The clinical characteristics of the 26 patients, who comprised 15 men and 11 women who were aged 32.07±9.29 years (range, 16-43 years) at the time of surgery, are listed in Table 1. The age at the onset of epilepsy was 11.11±6.98 years (range, 1-27 years), and the duration of epilepsy at the time of collection was 17.76±10.58 years (range, 1-42 years). All patients had complex partial seizures, and the frequency of seizures per month was 4.25±5.98 (range, 0.5-30). In 16 patients (61.5%), secondary generalization frequently occurred more than once per month. A history of childhood FS was reported in 13 patients (50%), and 7 patients reported other relevant causative childhood events (IPI), for example head trauma, meningitis, encephalitis, and Reye syndrome. No patient reported a history of SE. Most of the patients (80.7%) became seizure-free after their epilepsy surgery.

Pathological findings
The pathological grading scores of Nissl (for neuronal loss), nestin (for cell genesis), and Prox1 (for GCD) are listed in Table 2, based on the grading system presented in Fig. 1. Most patients showed a moderate-to-severe amount of neuronal loss: grade 3 in 12 patients (46.2%), grade 2 in 10 patients (38.5%), and grade 1 in 1 patient (3.8%). Most patients showed a mild-to-moderate amount of cell genesis: grade 1 in 8 patients (30.8%), grade 2 in 6 patients (23.1%), grade 3 in 11 patients (42.3%),
and grade 4 in 1 patient (3.8%). All patients exhibited some degree of GCD: grade 2 in 13 patients (50%), grade 3 in 7 patients (26.9%), and grade 4 in 6 patients (23.1%). With regard to the extrahippocampal pathological findings, only one patient exhibited apparent cortical dysplasia on magnetic resonance imaging (MRI) and macroscopic examination. However, 11 patients (42.3%) exhibited MRI-negative microscopic dysplasia in the resected temporal lobe specimens on histological examination.

**Association between clinical and pathological variables**

There were significant correlations between cell genesis and GCD ($r=0.43, p=0.03$) and between neuronal loss and GCD ($r=0.73, p<0.01$). Clinical variables, including age at onset, age at surgery, duration of epilepsy, and mean frequency of preoperative seizures, were not correlated with the pathological variables, including neuronal loss, cell genesis, and GCD (Table 3). We dichotomized the results of nestin immunoreactivity into a mild degree of cell genesis (grades 1 and 2) and a moderate-to-severe degree (grades 3 and 4). Moderate-to-severe cell genesis occurred more frequently in patients with a history of FS ($p=0.03$) (Fig. 2) and in those with frequent generalized seizures ($p=0.026$, both by chi-square test) (Fig. 2). Neuronal loss and GCD were not significantly correlated with FS or with the frequency of generalized seizures. The presence of temporal microscopic dysplasia was not significantly correlated with the other pathological findings, including neuronal loss, cell genesis, or GCD, or with any clinical variable including the history of FS and frequent generalized seizures.

**Discussion**

We investigated the histological changes (neuronal loss, cell genesis, and GCD) in the hippocampus from mTLE patients. Cell genesis, measured as the number of nestin-positive cells in the granule cell layer, was significantly correlated with the severity of GCD, history of childhood FS, and frequent gen-

---

**Table 1. Clinical parameters of medial temporal lobe epilepsy patients**

| Patient no. | Sex | Onset age (years) | Surgery age (years) | Epilepsy duration (years) | Seizure frequency (times/month) | Secondary generalization | FS | IPI | Age at IPI (years) |
|-------------|-----|------------------|--------------------|--------------------------|-------------------------------|------------------------|----|-----|------------------|
| 1           | F   | 1                | 43                 | 42                       | 8                             | F                      | –  | –   | 1                |
| 2           | M   | 4                | 23                 | 19                       | 3.5                           | R                      | +  | –   | 0                |
| 3           | F   | 4                | 37                 | 33                       | 4                             | F                      | –  | –   | 0                |
| 4           | F   | 16               | 28                 | 12                       | 8                             | F                      | +  | –   | 0                |
| 5           | M   | 14               | 27                 | 13                       | 0.5                           | R                      | +  | Head trauma  | 12               |
| 6           | F   | 11               | 19                 | 8                        | 5                             | R                      | –  | Reye syndrome  | 3                |
| 7           | F   | 1                | 18                 | 17                       | 1                             | F                      | +  | –   | 1                |
| 8           | M   | 13               | 21                 | 8                        | 0.5                           | F                      | +  | Meningitis  | 3                |
| 9           | M   | 27               | 39                 | 12                       | 2.5                           | F                      | –  | –   | 6                |
| 10          | M   | 6                | 20                 | 14                       | 30                            | F                      | +  | –   | 0                |
| 11          | M   | 16               | 51                 | 35                       | 1.5                           | F                      | –  | –   | 0                |
| 12          | M   | 11               | 37                 | 26                       | 10                            | R                      | –  | –   | 0                |
| 13          | F   | 12               | 16                 | 4                        | 1                             | F                      | +  | –   | 0                |
| 14          | M   | 15               | 27                 | 12                       | 1                             | F                      | +  | –   | 0                |
| 15          | M   | 20               | 31                 | 11                       | 1                             | F                      | –  | Encephalitis  | 10               |
| 16          | M   | 3                | 24                 | 21                       | 2                             | F                      | +  | –   | 3                |
| 17          | F   | 10               | 42                 | 32                       | 10                            | R                      | –  | –   | 0                |
| 18          | F   | 8                | 33                 | 25                       | 2                             | R                      | +  | –   | 0                |
| 19          | F   | 25               | 25                 | 1                        | 2                             | F                      | +  | –   | 0                |
| 20          | F   | 6                | 20                 | 14                       | 0.5                           | R                      | –  | –   | 0                |
| 21          | M   | 2                | 29                 | 27                       | 3                             | F                      | –  | –   | 2                |
| 22          | M   | 16               | 22                 | 6                        | ?                             | R                      | –  | –   | 0                |
| 23          | M   | 16               | 21                 | 5                        | 1                             | F                      | +  | –   | 0                |
| 24          | M   | 10               | 36                 | 26                       | 3.5                           | F                      | –  | –   | 0                |
| 25          | F   | 16               | 33                 | 17                       | 5                             | R                      | +  | –   | 0                |
| 26          | M   | 6                | 28                 | 22                       | 1                             | R                      | –  | Encephalitis  | 5                |

*Not all clinical data were available for every patient. For secondary generalization, F (frequent) means more than once per month and R (rare) means less than once per month.

M: male, F: female, FS: febrile seizures, IPI: initial precipitating injuries.*
eralized seizures. However, cell genesis was not associated with the extent of neuronal loss, age of seizure onset, duration of epilepsy, or the mean frequency of seizures in the preoperative period. In addition, the presence of microscopic cortical dysplasia in the surgical temporal lobe specimens was not correlated with other pathologic parameters including the degree of neuronal loss, cell genesis, and GCD. These results suggest that newly generated granule cells lead to GCD and are likely to be influenced by FS in childhood and by frequent episodes of generalized seizures.

Granule cell neurons are generated throughout life from a population of continuously dividing progenitor cells that reside in the subgranular zone of the rodent dentate gyrus.29 This also seems to occur in humans, because new neurons, as identified by bromodeoxyuridine staining, are generated from dividing progenitor cells in the dentate gyrus of the adult human brain.30 Prolonged seizure activity markedly increases neurogenesis in the dentate gyrus of adult rats. Pulse-chase bromodeoxyuridine labeling and immunohistochemistry for immature neuronal markers show that newly generated neurons migrate in chains from the dentate subgranular zone to ectopic locations in the hilus and molecular layer after pilocarpine-induced SE.26

Thus, one hypothesis is that GCD in humans with TLE results from enhanced neurogenesis induced by prolonged seizures. Although our results support this hypothesis, some recent studies have shown contrary results. For example, in a rodent model of epilepsy after kainate injection and in the hippocampi from patients with TLE, GCD does not result from increased neurogenesis, but rather from abnormal migration of mature granule cells along a radial glial scaffold, most likely caused by local reelin deficiency.15,31 Nestin, which is an indicator of cell genesis, is expressed in the neuroglial cells in addition to undifferentiated precursors of neurons.22 Therefore, nestin positivity cannot be attributed entirely to neurogenesis, and this may explain some of the differences between our data and those of previous studies.

We demonstrated increased cell genesis in patients with frequent generalized seizures. Likewise, seizure-induced neuro-

| Patient no. | Cell genesis (Nestin) | Neuronal loss (Nissl) | Granule cell dispersion (Prox1) | Cortical dysplasia in the temporal lobe surgical specimen |
|-------------|----------------------|----------------------|------------------|---------------------------------------------------|
| 1           | 1                    | 1                    | 2                | +                                                 |
| 2           | 2                    | 3                    | 3                | +                                                 |
| 3           | 2                    | 2                    | 2                | +                                                 |
| 4           | 1                    | 1                    | 2                | –                                                 |
| 5           | 3                    | 2                    | 2                | –                                                 |
| 6           | 1                    | 3                    | 3                | –                                                 |
| 7           | 3                    | 2                    | 2                | –                                                 |
| 8           | 2                    | 3                    | 3                | +                                                 |
| 9           | 3                    | 3                    | 4                | +                                                 |
| 10          | 3                    | 3                    | 2                | +                                                 |
| 11          | 3                    | 3                    | 4                | +                                                 |
| 12          | 3                    | 3                    | 4                | +                                                 |
| 13          | 3                    | 2                    | 3                | +                                                 |
| 14          | 3                    | 2                    | 3                | –                                                 |
| 15          | 3                    | 1                    | 2                | –                                                 |
| 16          | 3                    | 3                    | 4                | –                                                 |
| 17          | 1                    | 2                    | 2                | +                                                 |
| 18          | 2                    | 1                    | 2                | –                                                 |
| 19          | 2                    | 2                    | 2                | –                                                 |
| 20          | 1                    | 2                    | 2                | –                                                 |
| 21          | 3                    | 3                    | 3                | –                                                 |
| 22          | 1                    | 2                    | 2                | –                                                 |
| 23          | 3                    | 3                    | 4                | +                                                 |
| 24          | 4                    | 3                    | 4                | –                                                 |
| 25          | 2                    | 3                    | 3                | –                                                 |
| 26          | 1                    | 2                    | 2                | –                                                 |

Table 2. The pathological grading scores of Nissl staining, and nestin and Prox1 immunohistochemical, and the presence of cortical dysplasia in human temporal lobe surgical specimens

| Patient no. | Cell genesis (Nestin) | Neuronal loss (Nissl) | Granule cell dispersion (Prox1) | Cortical dysplasia in the temporal lobe surgical specimen |
|-------------|----------------------|----------------------|------------------|---------------------------------------------------|
|             |                      |                      |                  |                                                   |

Table 3. Pearson correlation coefficients between clinical variables and pathological results

|                         | Cell genesis (Nestin) | Neuronal loss (Nissl) | Granule cell dispersion (Prox1) |
|-------------------------|-----------------------|-----------------------|---------------------------------|
| Surgery age             | Correlation           | -0.06                 | -0.11                           | 0.16                            |
|                         | p                     | 0.76                  | 0.59                            | 0.45                            |
| Onset age               | Correlation           | 0.12                  | 0.01                            | 0.19                            |
|                         | p                     | 0.56                  | 0.97                            | 0.35                            |
| Epilepsy duration       | Correlation           | -0.1                 | -0.07                           | 0.04                            |
|                         | p                     | 0.61                  | 0.72                            | 0.85                            |
| Seizure frequency       | Correlation           | -0.11                | 0.13                            | -0.17                           |
|                         | p                     | 0.6                  | 0.52                            | 0.41                            |
| Cell genesis (Nestin)   | Correlation           | -                    | 0.32                            | 0.43                            |
|                         | p                     | -                    | 0.12                            | 0.03*                           |
| Neuronal loss (Nissl)   | Correlation           | 0.32                 | -                               | 0.73                            |
|                         | p                     | 0.12                 | -                               | <0.01*                          |
| Granule cell dispersion (Prox1) | Correlation | 0.43             | 0.73                            | -                               |
|                         | p                     | 0.03*                | <0.01*                          | -                               |

*Denotes significant correlation.
Neuropathologic Features of TLE

Fig. 2. The distribution of (A) patients with or without a history of FS and (B) with frequent or rare generalized seizures, relative to the degree of nestin positivity (cell genesis). A mild degree of cell genesis was classified as the pathological grading score of nestin of 1 or 2, and a moderate-to-severe degree of cell genesis was classified as grade 3 or 4. Cell genesis was significantly higher in patients with a history of FS (p = 0.030) and with frequent generalized seizures (p = 0.026). FS: febrile seizures.

Neurogenesis in the dentate gyrus has been proven in various animal models, such as, pilocarpine-induced SE,20,21,32 kainic acid injection,33 and amygdala kindling.34 Seizure-induced neurogenesis has also been described in adults16 and in children with mTLE.35,36 Although the role of seizure-induced neurogenesis in the pathophysiology of TLE is uncertain, it is possible that newly generated cells contribute to the formation of GCD and ectopic granule cells in the hilus. Seizure-induced cell proliferation and the likelihood of developing spontaneous recurrent seizures following pilocarpine-induced SE are reduced by the antimitotic agent cytosine-β-D-arabinofuranoside in adult rodent models, which suggests that hippocampal cell proliferation plays a proepileptic rather than a compensatory role.20

Experimentally prolonged FS results in late-onset limbic (temporal lobe) epilepsy,13 but the epileptogenic potential of prolonged FS in humans remains unclear. Although retrospective analysis has implicated early-life FS as a risk factor for the development of TLE in humans,38 whether early-life FS actually causes TLE or is simply indicative of another, perhaps genetically determined vulnerability that eventually results in TLE, cannot be determined in correlative clinical studies.37 Moreover, the epileptogenic mechanisms underlying FS remain unknown, but might involve enduring changes at the molecular and functional levels, such as alterations in neurotransmitter receptors or voltage-gated ion channels, and might not involve neuronal loss.39 Although the effects of FS on cell proliferation in the dentate gyrus have been described in animal models,40,41 there is little evidence of this in humans. We found a positive correlation between early-life FS and ongoing cell proliferation in adult human hippocampi taken from TLE patients, but further parallel human and animal studies are needed to demonstrate the role of altered cell proliferation after FS and other epileptogenic mechanisms of FS.

We found a positive correlation between the severity of HS (i.e., neuronal loss) and the presence of GCD, which is in accordance with the results of recent studies13,15 and with the original work by Houser.1 Considering that the presence of GCD was not correlated with clinical factors such as duration of epilepsy or frequency of seizures, GCD might be more closely linked to the pathological process of HS rather than being a manifestation of severe temporal lobe seizures.4 We also found no correlation between clinical variables relating to seizure burden and the severity of HS. This is a conflicting area, and most pathological or longitudinal MRI studies have suggested a correlation between the severity of hippocampal neuronal loss and the duration of epilepsy.15 However, few studies have thoroughly determined of the burden of seizures,13 and one limitation to our study is that we did not know the exact total number of generalized and partial seizures. Despite this limitation, we found no evidence that HS occurs as a consequence of recurrent seizures in patients with TLE.

Microscopic cortical dysplasia or microdysgenesis manifests as minor abnormalities on histological examination, even when the MRI or macroscopic examination reveals no abnormalities.42 Microscopic cortical dysplasia may appear in 20-45% of surgical specimens from mTLE patients with HS.43-45 We found microscopic cortical dysplasia in 11 patients (42.3%), a rate similar to that of previous reports, suggesting that it is a relatively commonly associated finding in HS. It has been suggested that this represents a preexisting susceptibility factor that renders the affected brain vulnerable to the development of mTLE after IPI or FS, but the reciprocal relationship remains unknown.44 Recent data show that microscopic cortical dysplasia is not related to clinical parameters such as IPI or FS, or to the histological characteristics of HS;43-45 our results are consistent with these previous findings. Hence, the role of microscopic cortical dysplasia is currently obscure, and future studies are needed to reveal the complexities.

The semiquantitative scoring system that we applied has
some limitations. We were unable to quantify real cell counts for neuronal loss and cell genesis, or to measure the real thickness of granule cell layer for GCD, because the tissue sections of the surgical specimens did not precisely correspond. Our results may thus be inconclusive; nevertheless, we think that our results might be suggestive of the neuropathologic features of human specimens.

In conclusion, our study showed that increased cell genesis is correlated with the severity of GCD in the human hippocampal dentate gyrus of medically intractable mTLE patients, supporting the view that newly generated granule cells might lead to GCD. The degree of cell genesis was also related to the history of childhood FS and frequent generalized seizures, but was not significantly associated with the degree of neuronal loss or other clinical variables, such as the age at onset, duration of epilepsy, or the mean frequency of all seizures.

Conflicts of Interest

The authors have no financial conflicts of interest.

Acknowledgements

This study was supported by the Ministry of Health and Welfare (no. A060452), Republic of Korea.

Dr. J-K. Roh was supported by a grant from Seoul National University Hospital, South Korea (no. 2120040140).

REFERENCES

1. Babb TL, Brown WJ. Pathological findings in epilepsy. In: Engel J Jr, New York: Raven Press; 1987:511-540.
2. Meencke HJ, Veith G. Hippocampal sclerosis in epilepsy. In: Luders H. Epilepsy surgery. New York: Raven Press; 1991:705-715.
3. Houser CR. Granule cell dispersion in the dentate gyrus of humans with temporal lobe epilepsy. Brain Res 1990:535:195-204.
4. Lurton D, El Bahh B, Sundstrom L, Rougier A. Granule cell dispersion is correlated with early epileptic events in human temporal lobe epilepsy. J Neurol Sci 1998;154:133-136.
5. El Bahh B, Lespinet V, Lurton D, Coussemacq M, Le Gal La Salle G, Rougier A. Correlations between granule cell dispersion, mossy fiber sprouting, and hippocampal cell loss in temporal lobe epilepsy. Epilepsia 1999;40:1393-1401.
6. Thom M, Sisodiya SM, Beckett A, Martinian L, Lin WR, Harkness W, et al. Cytoarchitectural abnormalities in hippocampal sclerosis. J Neuropathol Exp Neurol 2002;61:510-519.
7. Blümcke I, Thom M, Wiestler OD. Ammon’s horn sclerosis: a maldevelopmental disorder associated with temporal lobe epilepsy. Brain Pathol 2002;12:199-211.
8. Cavanagh JB, Meyer A. Aetiologic aspects of Ammon’s horn sclerosis associated with temporal lobe epilepsy. Br Med J 1956:2:1403-1407.
9. Dam AM. Epilepsy and neuron loss in the hippocampus. Epilepsia 1980; 21:617-629.
10. Babb TL, Brown WJ, Pretorius J, Davenport C, Lieb JP, Crandall PH. Temporal lobe volumetric cell densities in temporal lobe epilepsy. Epilepsia 1984:25:729-740.
11. Mathern GW, Adelson PD, Cahan LD, Leite JP. Hippocampal neuron damage in human epilepsy: Meyer’s hypothesis revisited. Prog Brain Res 2002;135:237-251.
12. Fuerst D, Shah J, Kupsky WJ, Johnson R, Shah A, Hayman-Abello B, et al. Volumetric MRI, pathological, and neuropsychological progression in hippocampal sclerosis. Neurology 2001;57:184-188.
13. Thom M, Zhou J, Martinian L, Sisodiya S. Quantitative post-mortem study of the hippocampus in chronic epilepsy: seizures do not inevitably cause neuronal loss. Brain 2005:128:1344-1357.
14. Jessberger S, Römer B, Babi H, Kempermann G. Seizures induce proliferation and dispersion of doublecortin-positive hippocampal progenitor cells. Exp Neurol 2005:196:342-351.
15. Fahrner A, Kann G, Flühacher A, Heinrich C, Freiman TM, Zentner J, et al. Granule cell dispersion is not accompanied by enhanced neurogenesis in temporal lobe epilepsy patients. Exp Neurol 2007;203:320-332.
16. Thom M, Martinian L, Williams G, Stoebker K, Sisodiya SM. Cell proliferation and granule cell dispersion in human hippocampal sclerosis. J Neuropathol Exp Neurol 2005:64:194-201.
17. Wiebe S, Blume WT, Girvin JP, Elasziw M. Effectiveness and Efficiency of Surgery for Temporal Lobe Epilepsy Study Group. A randomized, controlled trial of surgery for temporal-lobe epilepsy. N Engl J Med 2001;345:311-318.
18. Mathern GW, Babb TL, Vickrey BG, Melendez M, Pretorius JK. The clinical-pathogenic mechanisms of hippocampal neuron loss and surgical outcomes in temporal lobe epilepsy. Brain 1995;118:105-118.
19. Chu K, Kim M, Jung KH, Jeon D, Lee ST, Kim J, et al. Human neural stem cell transplantation reduces spontaneous recurrent seizures following pilocarpine-induced status epilepticus in adult rats. Brain Res 2004;1023:213-221.
20. Jung KH, Chu K, Kim M, Jeong SW, Song YM, Lee ST, et al. Continuous cytosine-b-D-arabinofuranoside infusion reduces ectopic granule cells in adult rat hippocampus with attenuation of spontaneous recurrent seizures following pilocarpine-induced status epilepticus. Eur J Neurosci 2004;19:3219-3226.
21. Jung KH, Chu K, Lee ST, Kim J, Sinn DI, Kim JM, et al. Cyclooxygenase-2 inhibitor, celecoxib, inhibits the altered hippocampal neurogenesis with attenuation of spontaneous recurrent seizures following pilocarpine-induced status epilepticus. Neurobiol Dis 2006:23:237-246.
22. Kruglyakova EP, Khovryakov AV, Shikhanov NP, MacCann GM 2nd, Vael Y, Kruglyakov PP, et al. Nestin-expressing cells in the human hippocampus. Neurosci Behav Physiol 2005;35:891-897.
23. Pleasure SJ, Collins AE, Lowenstein DH. Unique expression patterns of cell fate molecules delineate sequential stages of dentate gyrus development. J Neurosci 2000;20:6095-6105.
24. Elliott RC, Khademi S, Pleasure SJ, Parent JM, Lowenstein DH. Differential regulation of basic helix-loop-helix mRNAs in the dentate gyrus following status epilepticus. Neuroscience 2001;106:79-88.
25. Bagri A, Gurney T, He X, Zou YR, Littman DR, Tessier-Lavigne M, et al. The chemokine SDF1 regulates migration of dentate granule cells. Development 2002;129:4249-4260.
26. Parent JM, Elliott RC, Pleasure SJ, Barbaro NM, Lowenstein DH. aberrant seizure-induced neurogenesis in experimental temporal lobe epilepsy. Ann Neurol 2006;59:81-91.
27. McCloskey DP, Hintz TM, Pierce JP, Scharfman HE. Stereological methods reveal the robust size and stability of ectopic hilar granule cells after pilocarpine-induced status epilepticus in the adult rat. Eur J Neurosci 2006:24:2203-2210.
28. Pirttilä TJ, Manninen A, Jutila L, Nissinen J, Källvånnen R, Vapalahti M, et al. Cystatin C expression is associated with granule cell dispersion in epilepsy. Ann Neurol 2005;58:211-223.
29. Kuhn HG, Dickinson-Anson H, Gage FH. Neurogenesis in the dentate gyrus of the adult rat: age-related decrease of neuronal progenitor proliferation. J Neurosci 1996;16:2072-2033.
30. Eriksson PS, Perfilieva E, Björk-Eriksson T, Alborn AM, Nordborg C, Peterson DA, et al. Neurogenesis in the adult human hippocampus. Nat Med 1998;4:1313-1317.
31. Heinrich C, Nitta N, Fluhacher A, Muller M, Fahrner A, Kirsch M, et al. Reelin deficiency and displacement of mature neurons, but not neurogenesis, underlie the formation of granule cell dispersion in the epi...
Neuropathologic Features of TLE

32. Scharffman HE, Goodman JH, Sollas AL. Granule-like neurons at the hilar/CA3 border after status epilepticus and their synchrony with area CA3 pyramidal cells: functional implications of seizure-induced neurogenesis. J Neurosci 2000;20:6144-6158.

33. Gray WP, Sundstrom LE. Kainic acid increases the proliferation of granule cell progenitors in the dentate gyrus of the adult rat. Brain Res 1998;790:52-59.

34. Parent JM, Janumpalli S, McNamara JO, Lowenstein DH. Increased dentate granule cell neurogenesis following amygdala kindling in the adult rat. Neurosci Lett 1998;247:9-12.

35. Bäumcke I, Scheewe JC, Normann S, Brüstle O, Schramm J, Elger CE, et al. Increase of nestin-immunoreactive neural precursor cells in the dentate gyrus of pediatric patients with early-onset temporal lobe epilepsy. Hippocampus 2001;11:311-321.

36. Takei H, Wilfong A, Yoshor D, Armstrong DL, Bhattacharjee MB. Evidence of increased cell proliferation in the hippocampus in children with Ammon's horn sclerosis. Pathol Int 2007;57:76-81.

37. Dube C, Richichi C, Bender RA, Chung G, Litt B, Baram TZ. Temporal lobe epilepsy after experimental prolonged febrile seizures: prospective analysis. Brain 2006;129:911-922.

38. Cendes F, Andermann F. Do febrile seizures promote temporal lobe epilepsy? Retrospective studies. In: Baram TZ, Shinnar S. San Diego: Academic Press, 2002;78-88.

39. Dube CM, Brewster AL, Richichi C, Zha Q, Baram TZ. Fever, febrile seizures and epilepsy. Trends Neurosci 2007;30:490-496.

40. Lemmens EM, Lubbers T, Schijns OE, Beuls EA, Hoogland G. Gender differences in febrile seizure-induced proliferation and survival in the rat dentate gyrus. Epilepsia 2005;46:1603-1612.

41. Bender RA, Dube C, Gonzalez-Vega R, Minn EW, Baram TZ. Mossy fiber plasticity and enhanced hippocampal excitability, without hippocampal cell loss or altered neurogenesis, in an animal model of prolonged febrile seizures. Hippocampus 2003;13:399-412.

42. Armstrong DD. The neuropathology of temporal lobe epilepsy. J Neuropathol Exp Neurol 1993;52:433-443.

43. Kasper BS, Stefan H, Paulus W. Microdysgenesis in mesial temporal lobe epilepsy: a clinicopathological study. Ann Neurol 2003;54:501-506.

44. Diehl B, Najm I, LaPresto E, Prayson R, Ruggieri P, Mohamed A, et al. Temporal lobe volumes in patients with hippocampal sclerosis with or without cortical dysplasia. Neurology 2004;62:1729-1735.

45. Kalnins RM, McIntosh A, Saling MM, Berkovic SF, Jackson GD, Briellmann RS. Subtle microscopic abnormalities in hippocampal sclerosis do not predict clinical features of temporal lobe epilepsy. Epilepsia 2004;45:940-947.