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Efficacy of the Ketogenic Diet for Pediatric Epilepsy According to the Presence of Detectable Somatic mTOR Pathway Mutations in the Brain

Ara Ko[a], Nam Suk Sim[b]
Han Som Choi[c,d], Donghwa Yang[e]
Se Hee Kim[d], Joon Soo Lee[d]
Dong Seok Kim[f], Jeong Ho Lee[b]
Heung Dong Kim[d]
Hoon-Chul Kang[d]

[a]Department of Pediatrics, Pusan National University Children's Hospital, Pusan National University School of Medicine, Yangsan, Korea
[b]Graduate School of Medical Science and Engineering, Korea Advanced Institute of Science and Technology (KAIST), Daejeon, Korea
[c]Department of Pediatrics, Ewha Womans University Seoul Hospital, Ewha Womans University School of Medicine, Seoul, Korea
[d]Division of Pediatric Neurology, Department of Pediatrics, Severance Children's Hospital, Yonsei University College of Medicine, Seoul, Korea
[e]Department of Pediatrics, National Health Insurance Service Ilsan Hospital, Goyang, Korea
[f]Department of Neurosurgery, Pediatric Neurosurgery, Severance Children's Hospital, Yonsei University College of Medicine, Seoul, Korea

Background and Purpose A multifactorial antiepileptic mechanism underlies the ketogenic diet (KD), and one of the proposed mechanisms of action is that the KD inhibits the mammalian target of rapamycin (mTOR) pathway. To test this clinically, this study aimed to determine the efficacy of the KD in patients with pathologically confirmed focal cortical dysplasia (FCD) due to genetically identifiable mTOR pathway dysregulation.

Methods A cohort of patients with pathologically confirmed FCD after epilepsy surgery and who were screened for the presence of germline and somatic mutations related to the mTOR pathway in peripheral blood and resected brain tissue was constructed prospectively. A retrospective review of the efficacy of the prior KD in these patients was performed.

Results Twenty-five patients with pathologically confirmed FCD and who were screened for the presence of detectable somatic mTOR pathway mutations had received a sufficient KD. Twelve of these patients (48.0%) had germline or somatic detectable mTOR pathway mutations. A response was defined as a ≥50% reduction in seizure frequency. The efficacy of the KD after 3 months of dietary therapy was superior in patients with detectable mTOR pathway mutations than in patients without detectable mTOR pathway mutations, although the difference was not statistically significant (responder rates of 58.3% vs. 38.5%, p=0.434).

Conclusions A greater proportion of patients with mTOR pathway responded to the KD, but there was no statistically significant difference in efficacy of the KD between patients with and without detectable mTOR pathway mutations. Further study is warranted due to the smallness of the sample and the limited number of mTOR pathway genes tested in this study.

Keywords mammalian target of rapamycin; epilepsy; focal cortical dysplasia; mTORopathies; somatic mutation; ketogenic diet.

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Correspondence
Hoon-Chul Kang, MD, PhD
Department of Pediatrics, Severance Children's Hospital, Yonsei University College of Medicine, 50-1 Yonsei-ro, Seodaemun-gu, Seoul 03722, Korea
Tel +82-2-2228-2061
Fax +82-2-2228-2056
E-mail hipo0207@yuhs.ac

Heung Dong Kim, MD, PhD
Department of Pediatrics, Severance Children's Hospital, Yonsei University College of Medicine, 50-1 Yonsei-ro, Seodaemun-gu, Seoul 03722, Korea
Tel +82-2-2228-2061
Fax +82-2-2228-2056
E-mail hdkimmd@yuhs.ac

INTRODUCTION

The ketogenic diet (KD) is characterized by high fat, adequate protein, and low carbohydrate contents, and is an established and effective therapy used since 1921 for drug-resistant epilepsy.[1,2] Randomized controlled studies have shown that children with drug-resistant epilepsy have significantly fewer seizures (seizure frequency reduction of ≥50%) at 3−4 months after consuming the KD than those not on the KD.[3-5] Besides its antiepileptic effect, the KD has also been shown to have disease-modifying and antiepileptogenic properties.[6,7]

Despite the rapid increase in its clinical use in recent years, the mechanism of action of the KD is still not fully understood. One of the numerous suggested hypotheses is that the KD inhibits the mammalian target of rapamycin (mTOR) pathway.[8] McDaniel et al.[8] found that rats on a KD had decreased expression levels in the hippocampus and liver of phosphory-

lated S6 (pS6) and phosphorylated AKT (pAKT), which are markers for the activation of mTOR complex 1 (mTORC1) and mTOR complex 2, respectively. Based on this result, those authors suggested that the KD exerts antiepileptic or antiepileptogenic effects via inhibition of the mTOR pathway.[8]

Focal cortical dysplasia (FCD) is an important cause of drug-resistant epilepsy in children, and is the most frequent etiology for epilepsy surgery in children.[9] FCD type II—referring to an isolated lesion characterized by cortical dyslamination and dysmorphic neurons without (FCD type IIa) or with (FCD type IIb) balloon cells—is the most common pathological finding in tissues resected from patients who undergo epilepsy surgery due to drug-resistant epilepsy, with FCD type IIb being the most frequent.[10,11] There is increasing evidence in the literature from molecular studies linking FCD type II to hyperactivation of the mTOR pathway.[12-14] Accordingly, brain somatic mutations in genes of the mTOR pathway have been shown to play a major role in the etiology of FCD type II.[15-17]

Based on the assumption that the antiepileptic effect of the KD is due to mTOR pathway inhibition, this study aimed to determine the efficacy of the KD in patients with pathologically confirmed FCD due to genetically identifiable mTOR pathway dysregulation.

METHODS

Patients

A prospective cohort was constructed of patients with pathologically confirmed FCD after epilepsy surgery at Severance Children's Hospital. The patients were thoroughly screened for the presence of somatic mutations related to the mTOR pathway. We then performed a retrospective review of the efficacy of the KD in these patients (KD prior to this study was investigated retrospectively, and the mTOR assessment was performed prospectively). The inclusion criteria for this study were as follows: 1) received epilepsy surgery at Severance Children's Hospital since 2004, 2) had FCD as pathologically confirmed in brain tissue samples, 3) assessed for low-level somatic mutations of mTOR pathway genes by deep sequencing of brain tissue samples, and 4) consumed the KD for more than 3 months or discontinued the KD before 3 months due to inefficacy as determined by an epileptologist. Patients who discontinued the KD before 3 months due to reasons other than inefficacy, such as poor compliance, adverse events, or proceeding to second surgery, were excluded. In short, we investigated the effectiveness of the past KD before the surgery for patients with confirmed FCD by pathology.

The KD was applied to 29 of 84 patients with pathologically confirmed FCD after epilepsy surgery and whose brain tissue samples were evaluated using deep sequencing. Four patients had discontinued the KD before 3 months: two before receiving epilepsy surgery, one due to poor compliance, and one due to recurrent vomiting resulting in weight loss. The remaining 25 patients were included and assessed in this study.

This study was approved by the Institutional Review Board of Severance Children's Hospital and KAIST (IRB No 4-2011-0822). All human tissues were obtained with informed consent in accordance with protocols approved by Severance Children's Hospital and the KAIST Institutional Review Board and Committee on Human Research.

Detection of low-level somatic mutations: DNA extraction from brain tissue samples and genetic analysis

Genomic DNA was extracted from peripheral blood samples using the QIAamp DNA Blood Mini Kit (Qiagen, Valencia, CA, USA), and from brain tissue samples using the QIAamp DNA Mini Kit (Qiagen, Valencia, CA, USA) for fresh-frozen tissue and the QIAamp DNA FFPE Tissue Kit (Qiagen, Valencia, CA, USA) for formalin-fixed, paraffin-embedded tissue.

Genetic analysis was conducted as described previously.[17-19] Briefly, we performed whole-exome sequencing for 2 patients (average read depth, 240×) and hybrid capture sequencing targeting 6 genes (*MTOR*, *PIK3CA*, *PIK3R2*, *AKT3*, *TSC1*, and *TSC2*) for 10 patients (average read depth, 633×). Another hybrid capture sequencing targeting 28 genes (*MTOR*, *AKT3*, *PIK3CA*, *PIK3R2*, *PTEN*, *STRADA*, *TSC1*, *TSC2*, *DEPDC5*, *SCN1A*, *CACNA1A*, *GRIN1*, *GABRA1*, *GABRB3*, *GABBR2*, *DCX*, *CHD2*, *LIS1*, *TUBB2B*, *FASN*, *FLNA*, *BRAF*, *EZH2*, *HNRNPU*, *IQSEC2*, *NEDD4L*, *ALG13*, and *DNM1*) was conducted for 13 patients (average read depth, 1,256×). All sequencing data were aligned to the GRCh37 reference and preprocessed as described in the best-practices workflow suggested by the Broad Institute (https://software.broadinstitute.org/gatk/best-practices/). Somatic mutations found only in the brain were identified using Mutect, Strelka, and Mutect2 variant callers, which can analyze paired blood–brain sequencing data sets.[20,21] All identified variants were annotated using the snpEFF program.[22] We then applied the following exclusion criteria: 1) registered mutations in a public database (common dbSNP147); 2) mutations with a putative low snpEFF impact score; 3) mutations with PolyPhen and SIFT scores indicating that they are not damaging, and a phastCons score of <0.9; and 4) mutations with an allele frequency of >0.1% in the ExAC database for minor allele frequencies and East Asian populations.[23] After applying the filtering process, all candidate variants were validated using targeted amplicon sequencing.

Ketogenic diet

Patients were instructed to follow a classic 4:1 KD, classic 3:1 KD, or modified Atkins diet by the attending pediatric epileptologists.[24] Patients immediately began the diet regimen without an initial fasting period, and calories were restricted to 75% of the recommended daily intake. Screening and follow-up examinations were performed according to the protocol reported by Kang et al.,[25] and included measurements of serum β-hydroxybutyrate and urine ketone bodies to assess ketosis and adjust the ratios if required. The KD ratio that was maintained for the longest period during the first 3 months was selected for analysis.

Assessment of KD efficacy

Seizure frequencies were obtained before KD initiation (baseline) and after 3 and 6 months on the diet. To assess seizure frequency, the number of seizures that had occurred during the previous month was counted. Patients were considered KD responders if they showed a seizure frequency reduction of ≥50% relative to the baseline, and nonresponders otherwise. The proportion of patients with a seizure frequency reduction of ≥90% was also calculated.

Patients were included as nonresponders if they discontinued the KD before the time of the assessment due to inefficacy as determined by an epileptologist. The patients who discontinued the KD before 6 months for other reasons were excluded at the 6-month assessment.

Assessment of surgical outcome

The surgical outcome was assessed after 2 years of epilepsy surgery according to the following classification of the International League Against Epilepsy (ILAE) for epileptic seizures following epilepsy surgery:[26] 1, completely seizure-free; 2, only auras; 3, one to three seizure days per year; 4, four seizure days per year to seizure frequency reduction of ≥50%; 5, seizure frequency reduction of <50% to 100% increase in seizure days relative to the baseline; and 6, >100% increase in seizure days relative to the baseline.[26]

Statistical analysis

Data are expressed as median and interquartile range (IQR) values or as number and percentage values. Comparisons of KD efficacy between two groups were performed using chi-square tests or Fisher's exact tests for categorical data and Mann-Whitney U-tests for continuous data. Statistical significance was set at a p value less than 0.05. The Statistical Package for the Social Sciences software (IBM SPSS Statistics version 25; IBM Corp., Armonk, NY, USA) was used for the statistical analyses.

RESULTS

The patients with FCD pathologically confirmed after resective epilepsy surgery included 29 who had consumed the KD before the epilepsy surgery and received deep sequencing for the presence of mTOR pathway mutations in their brain tissue samples obtained during the surgery. Four patients who discontinued the KD before 3 months due to reasons other than inefficacy were excluded, and data on the remaining 25 patients were analyzed (Fig. 1). The median age at KD initiation was 4.1 years (IQR, 2.2–5.0 years; range, 0.6–15.5 years). All patients showed daily seizures at KD initiation, had taken at least two antiepileptic drugs previously, and exhibited drug-resistant epilepsy. The median age at seizure onset was 0.5 years (IQR, 0.2–1.9 years), and the median duration from seizure onset to the KD (lead time of KD) was 2.8 years (IQR, 1.1–4.6 years). The KD was maintained for a median duration of 6.3 months (IQR, 3.4–11.4 months). At initiation, 18 (72.0%) patients consumed the 4:1 KD, 4 (16.0%) consumed the 3:1 KD, and 3 (12.0%) consumed the modified Atkins diet. Adequate ketosis as verified by measurements of serum β-hydroxybutyrate and urine ketone bodies was achieved in all patients. The syndromic diagnosis was unspecified focal epilepsy in 13 (52.0%) patients, Lennox-Gastaut syndrome in 10 (40.0%) patients, and West syndrome in 2 (8.0%) patients. The pathological classification of FCD was type IIa in 17 (68.0%) patients and type IIb in 8 (32.0%) patients. The clinical characteristics of the patients included in this study are summarized in Table 1.

The 25 patients included 12 (48.0%) with germline mTOR pathway mutations (1 patient, *DEPDC5*) or somatic detectable mTOR pathway mutations (11 patients; 8 *MTOR*, 2 *TSC1*, and 1 *TSC2*). None of the clinical characteristics (age at seizure onset, age at KD initiation, sex, baseline seizure frequency, number of antiepileptic drugs taken, syndromic diagnosis, classification of FCD, lead time of KD, KD duration, or KD ratio) differed significantly between patients with and without detectable mTOR pathway mutations.

One patient discontinued the KD during the first 3 months due to inefficacy and so was counted as a nonresponder. Between 3 and 6 months, three more patients discontinued the KD due to inefficacy and so they were also included as nonresponders at the 6-month efficacy analysis. No patients discontinued the KD between 3 and 6 months due to other reasons. Twelve (48.0%) patients showed a response (≥50% seizure frequency reduction relative to the baseline) after 3 months on the KD. To investigate whether clinical factors other than the presence of detectable mTOR pathway mutations affected KD efficacy, the clinical variables listed in Table 1 were compared between responders and nonresponders to the KD after 3 months. None of the clinical variables differed signifi-

cantly between responders and nonresponders to the KD at 3 months.

KD efficacy was assessed according to the presence of detectable mTOR pathway mutations. Seven (58.3%) patients with and five (38.5%) patients without detectable mTOR pathway mutations were responders to the KD (≥50% seizure fre-

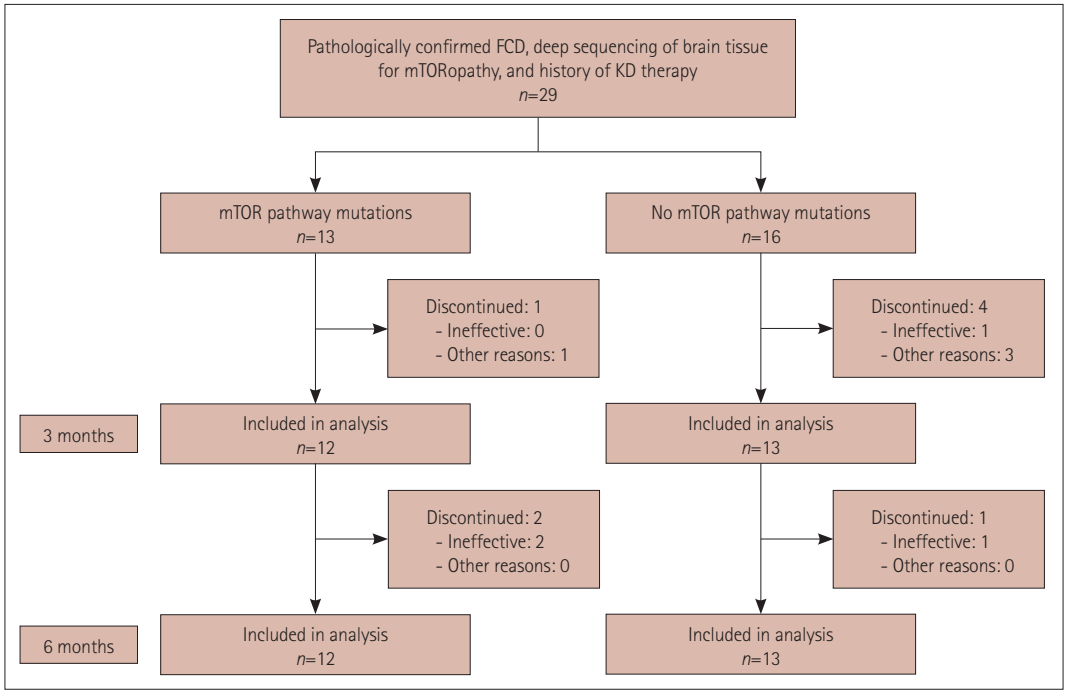

Fig. 1. Patient inclusion profile. Patients who discontinued the KD prematurely due to inefficacy as determined by an epileptologist were included in the analyses and classified as nonresponders. Patients who discontinued the KD due to other reasons were excluded from the analyses. FCD, focal cortical dysplasia; KD, ketogenic diet; mTOR, mammalian target of rapamycin.

Table 1. Clinical characteristics of patients with and without detectable mTORopathies

Clinical variables	Total (*n*=25)	mTOR pathway mutations detected (*n*=12)	No mTOR pathway mutations detected (*n*=13)	*p*
Sex, male	17 (68.0)	6 (50.0)	11 (84.6)	0.097
Age at seizure onset (yr)	0.5 (0.2–1.9)	1.1 (0.2–4.1)	0.3 (0.1–0.8)	0.270
Age at KD initiation (yr)	4.1 (2.2–5.0)	4.4 (2.6–6.5)	3.9 (1.3–4.9)	0.347
Lead time of KD (yr)	2.8 (1.1–4.6)	2.9 (1.0–4.7)	2.8 (1.0–4.4)	0.852
KD duration (month)	6.3 (3.4–11.4)	6.1 (3.5–10.7)	6.9 (2.3–13.2)	0.483
Diet				0.772
Classic 4:1 KD	18 (72.0)	8 (66.7)	10 (76.9)	
Classic 3:1 KD	4 (16.0)	2 (16.7)	2 (15.4)	
Modified Atkins diet	3 (12.0)	2 (16.7)	1 (7.7)	
Baseline seizure frequency before KD (per day)	13.0 (5.0–42.5)	5.0 (3.5–33.3)	20.0 (10.5–52.5)	0.060
Number of AEDs taken before KD	3 (2–4)	3 (3–4)	3 (2–4)	0.574
Syndromic diagnosis at time of KD initiation				0.217
Focal epilepsy, unspecified	13 (52.0)	8 (66.7)	5 (38.5)	
Lennox-Gastaut syndrome	10 (40.0)	4 (33.3)	6 (46.2)	
West syndrome	2 (8.0)	0 (0.0)	2 (15.4)	
FCD classification				0.097
Type IIa	17 (68.0)	6 (50.0)	11 (84.6)	
Type IIb	8 (32.0)	6 (50.0)	2 (15.4)	

Data are number (percentage) or median (interquartile range) values.
AEDs, antiepileptic drugs; FCD, focal cortical dysplasia; KD, ketogenic diet; mTOR, mammalian target of rapamycin.

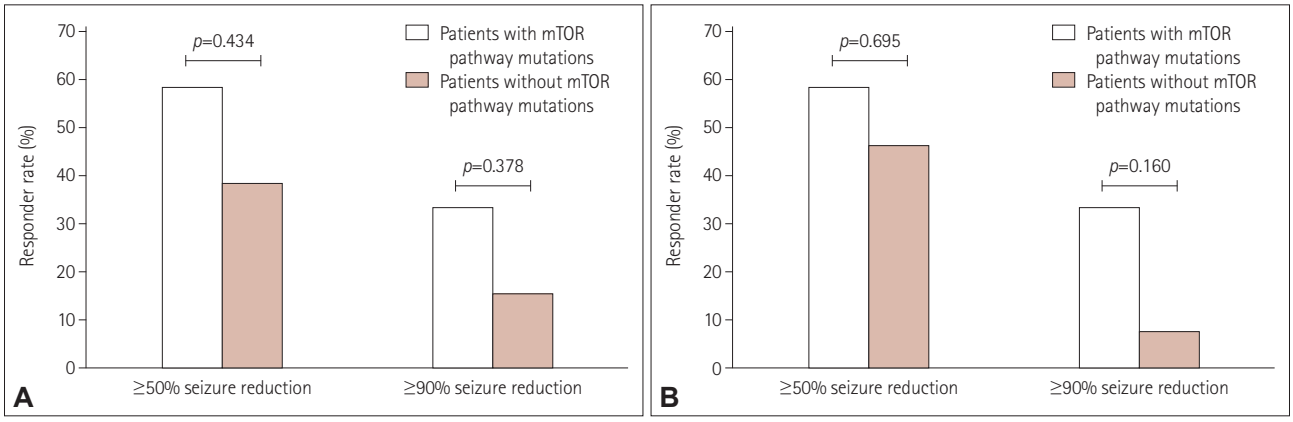

Fig. 2. Comparison of KD efficacy between patients with and without detectable mTOR pathway mutations. A: Outcome after 3 months. A seizure frequency reduction of ≥50% was achieved in 58.3% of patients with and 38.5% of patients without detectable mTOR pathway mutations (p=0.434). A seizure frequency reduction of ≥90% was achieved in 33.3% of patients with and 15.4% of patients without detectable mTOR pathway mutations (p=0.378). B: Outcome after 6 months. A seizure frequency reduction of ≥50% was achieved in 58.3% of patients with and 46.2% of patients without detectable mTOR pathway mutations (p=0.695). A seizure frequency reduction of ≥90% was achieved in 33.3% of patients with and 7.7% of patients without detectable mTOR pathway mutations (p=0.160). KD, ketogenic diet; mTOR, mammalian target of rapamycin.

quency reduction relative to the baseline) (Fig. 2A); however, the difference was not statistically significant (p=0.434). Additionally, four (33.3%) patients with and two (15.4%) patients without detectable mTOR pathway mutations showed ≥90% seizure frequency reduction relative to the baseline; this difference was also not statistically significant (p=0.378).

After 6 months on the KD, 13 (52.0%) patients showed a response (≥50% seizure frequency reduction relative to the baseline): 7 (58.3%) of these patients had and 6 (46.2%) did not have detectable mTOR pathway mutations (Fig. 2B); however, this difference was not statistically significant (p=0.695). Additionally, 4 (33.3%) patients with and 1 (7.7%) patient without detectable mTOR pathway mutations showed ≥90% seizure frequency reduction relative to the baseline; this difference also did not reach statistical significance (p=0.160).

The 21 patients who maintained the KD for ≥6 months comprised 13 and 2 who discontinued the KD at 6–9 months and 9–12 months, respectively, and 6 who continued the KD for more than 1 year. Among the 13 responders at 6 months, 4 patients discontinued the KD due to adverse events (2 due to recurrent vomiting and 2 due to food refusal), 3 patients discontinued without consulting their treating physicians (noncompliance), 4 patients discontinued before epilepsy surgery, and 2 patients discontinued after being seizure-free for 2 years. The four patients who discontinued the KD before their epilepsy surgery had a ≥50% seizure frequency reduction relative to the baseline at the time of discontinuation, and two patients who discontinued the KD after 2 years showed seizure recurrence at 4 and 9 months after KD discontinuation, respectively, leading to epilepsy surgery.

At 2 years after the epilepsy surgery, 16 (64.0%), 4 (16.0%), 2 (8.0%), 1 (4.0%), and 2 (8.0%) patients belonged to ILAE epilepsy surgical outcome classifications of 1, 3, 4, 5, and 5, respectively. The rate of achieving seizure freedom (ILAE surgical outcome classification of 1) did not differ between the patients with and without detectable mutations in genes of the mTOR pathway (58.3% vs 69.2%, p=0.688).

DISCUSSION

This study found that the response to the KD tended to be better in patients with detectable mTOR pathway mutations; however, the difference did not reach statistical significance. This result suggests that inhibition of the mTOR pathway is not a strong mechanism underlying the antiepileptic effect of the KD.

The increased clinical interest in the KD has prompted a recent rapid increase in research into the mechanisms of action of this diet. One of the suggested mechanisms is the alteration of neurotransmitter levels by the KD, resulting in increased levels of γ-aminobutyric acid or adenosine, or decreased levels of glutamate.[27-29] Additionally, the KD may exert an antiseizure effect by increasing mitochondrial biogenesis, reducing reactive oxygen species production via enhancing the expression of uncoupling proteins, or increasing resistance to oxidative stress by inhibition of class I histone deacetylases.[30-33] Other hypotheses on the antiepileptic mechanisms of the KD include reduced glycolysis, increased levels of polyunsaturated fatty acids, increased ATP production through increased anaplerosis, inhibition of lactate dehydrogenase, and an anti-inflammatory effect induced by KD.[34-42] However, other studies have produced evidence that contradicts these hypotheses, and so the mechanisms of action of the KD are still unclear.[43-45]

Another suggested mechanism for the antiepileptic and an-

tiepileptogenic effects of the KD is inhibition of the mTOR pathway.[8] The mTOR is a ubiquitous protein that regulates cell growth, proliferation, metabolism, protein synthesis, and autophagy.[46] In the brain, the mTOR mediates neurogenesis, migration, neuronal and glial differentiation, axonal sprouting and regeneration, myelination, dendritic development, and glial function.[46-51] The term "mTORopathies" was recently coined to define a continuum of neurological disorders characterized by altered cortical architecture, abnormal neuronal morphology, and intractable seizures as a consequence of abnormal mTOR signaling.[52] Dysregulation of the mTOR pathway caused by mechanisms such as gain-of-function mutations of *MTOR* or loss-of-function mutations of genes encoding for mTOR inhibitors (e.g., *TSC1*, *TSC2*, *PTEN*, and *DEPDC5*) might induce neuronal excitability by modulating the expression levels of ion channels and receptors.[53-56] Although the exact mechanisms of epileptogenesis associated with a dysregulated mTOR pathway are not yet fully explained, an association between dysregulated mTOR signaling and epilepsy has been identified in various models of genetic and acquired epilepsy due to the tuberous sclerosis complex, FCD, traumatic brain injury, neonatal hypoxic ischemic injury, and pilocarpine- or kainic-acid–induced status epilepticus.[57-62] The mTOR inhibitor rapamycin has been shown to prevent the development of epilepsy in animal models of the aforementioned conditions, further supporting a role of the mTOR in epileptogenesis.[59,60,63-65] Therefore, the hypothesis of the ability of the KD to inhibit the mTOR pathway led to promising predictions of seizure control in patients with mTORopathies.[8] The KD was found to reduce pS6 and pAKT expression levels in the hippocampus of normal rats, while pS6 expression levels in the hippocampus and neocortex were lower in rats fed the KD than in those fed a regular diet at 7 and 21 days after status epilepticus was induced by kainic acid.[8] In another study, the KD with medium-chain fatty acids also inhibited the mTOR signaling pathway by decreasing the expression levels of AMPK, MTORC1, and P70SK in mice.[66] KD similarly inhibited the mTOR signaling pathway by decreasing the expression levels of AMPK, pS6, and p4EBP1 in Kcna1-null mice with increased mTOR signaling, and also reduced the severity of seizures.[67]

Based on these previous studies, we constructed a specific cohort of patients with pathologically confirmed FCD who were screened for low-level somatic mutations in order to test the hypothesis that patients with mTOR pathway-related mutations respond better to the KD than do patients without such mutations. However, the result of this study was not as expected. Despite the tendency toward a better response to the diet, patients with detectable mTOR pathway mutations did not show a significantly superior response to the KD than did those without detectable mTOR pathway mutations. There are some possible explanations for this negative result. This study only included patients with drug-resistant epilepsy due to FCD who received resective surgeries, in order for the cohort to be a more homogeneous and definitive. This restricted subgroup of mTORopathies might not reflect the entirety of epilepsy conditions induced by mTORopathies, which could explain the reduced efficacy of the KD found in this study. More importantly, unlike the animal model where the diet was introduced immediately after the cessation of status epilepticus, in this study the KD was initiated in patients with mTORopathies at a median duration of 2.9 years (IQR, 1.0–4.7 years) after seizure onset.[8] Such a longstanding epileptic condition may be more difficult to reverse by the KD, especially as the seizures themselves have been shown to cause mTOR activation; this may also be the reason for the reduced efficacy of the KD in patients with detectable mTORopathies found in this study.[62] Future trials are therefore necessary to determine whether mTOR inhibition by the KD can truly alter the clinical course by preventing epileptogenesis and modifying altered dynamics in epilepsy patients with mTORopathies, rather than by simply suppressing their seizures.

This study compared the efficacy of the KD between patients who had relatively homogeneous clinical characteristics with the exception of the presence or absence of detectable mTOR pathway mutations identified by deep sequencing designed to detect low-level somatic mutations. However, it had noteworthy limitations, including the small number of included patients and the focus being restricted to drug-resistant epilepsy patients with FCD in order to reflect patients with mTORopathies. Also, most (23/25, 92.0%) of the patients included in this study were evaluated with a targeted next-generation sequencing panel covering only a portion of the genes associated with mTOR pathway, and only two patients underwent whole-exome sequencing. This may have led to the inclusion of undetected patients with mTOR pathway defects in the mutation-negative group, especially as there are previous reports showing mTOR pathway hyperactivation in FCD patients whose causative mutations were not identified.[68]

In conclusion, the efficacy of the KD did not differ significantly between patients with and without detectable mTOR pathway mutations, although there was a tendency for a slightly larger proportion of the patients with detectable mTOR pathway mutations to respond to the KD. Further investigations are warranted due to the smallness of the sample and the small number of mTOR pathway genes tested in this study.

Availability of Data and Material

The datasets generated or analyzed during the study are available from the corresponding author on reasonable request.

ORCID iDs

Ara Ko https://orcid.org/0000-0002-3008-8432
Nam Suk Sim https://orcid.org/0000-0002-5567-8076
Han Som Choi https://orcid.org/0000-0002-5818-7985
Donghwa Yang https://orcid.org/0000-0002-1580-9406
Se Hee Kim https://orcid.org/0000-0001-7773-1942
Joon Soo Lee https://orcid.org/0000-0001-9036-9343
Dong Seok Kim https://orcid.org/0000-0001-8210-170X
Jeong Ho Lee https://orcid.org/0000-0002-2299-630X
Heung Dong Kim https://orcid.org/0000-0002-8031-7336
Hoon-Chul Kang https://orcid.org/0000-0002-3659-8847

Author Contributions

Conceptualization: all authors. Data curation: Ara Ko, Nam Suk Sim. Formal analysis: Ara Ko, Nam Suk Sim. Funding acquisition: Hoon-Chul Kang. Investigation: Ara Ko, Nam Suk Sim, Han Som Choi, Donghwa Yang. Methodology: Ara Ko, Nam Suk Sim, Han Som Choi, Donghwa Yang. Supervision: Jeong Ho Lee, Hoon-Chul Kang, Heung Dong Kim. Validation: all authors. Writing—original draft: Ara Ko. Writing—review & editing: Hoon-Chul Kang, Heung Dong Kim.

Conflicts of Interest

Hoon-Chul Kang, an assoicate editor of the *Journal of Clinical Neurology,* was not involved in the editorial evaluation or decision to publish this article. All remaining authors have declared no conflicts of interest.

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