FULL-LENGTH ORIGINAL RESEARCH

Use of the ketogenic diet to manage refractory epilepsy in CDKL5 disorder: Experience of >100 patients

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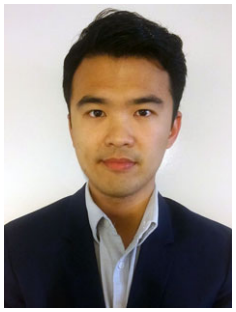

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Summary

Objective: **Pathogenic variants involving the *CDKL5* gene result in a severe epileptic encephalopathy, often later presenting with features similar to Rett syndrome. Cardinal features of epilepsy in the CDKL5 disorder include early onset at a median age of 6 weeks and poor response to antiepileptic drugs. The ketogenic diet (KD) was first introduced in the 1920s as a treatment option for refractory epilepsy in children. This study investigated use of the KD in the CDKL5 disorder and its influences on seizures.**

Methods: **The International CDKL5 Disorder Database, established in 2012, collects information on individuals with the CDKL5 disorder. Families have provided information regarding seizure characteristics, use, and side effects of the KD treatment. Descriptive statistics and time to event analyses were performed. Clinical vignettes were also provided on patients attending Boston Children's Hospital.**

Results: **Data regarding KD use were available for 204 individuals with a pathogenic *CDKL5* variant. Median age of inclusion in the database was 4.8 years (range = 0.3–33.9 years), with median age of 6 weeks (range = 1 day–65 weeks) at seizure onset. History of KD use was reported for 51% (104 of 204) of individuals, with a median duration of use of 17 months (95% confidence interval = 9–24). Changes in seizure activity after commencing KD were reported for two-thirds (69 of 104), with improvements in 88% (61 of 69). Nearly one-third (31.7%) experienced side effects during the diet. At ascertainment, only one-third (32%) remained on the diet, with lack of long-term efficacy as the main reason for diet cessation (51%, 36 of 70).**

Significance: **Benefits of KD in the CDKL5 disorder are in keeping with previous trials on refractory epilepsies. However, poor long-term efficacy remains as a significant barrier. In view of its side effect profile, KD administration should be supervised by a pediatric neurologist and specialist dietician.**

KEY WORDS: **Ketogenic diet, CDKL5 disorder, Refractory epilepsy, Cyclin-dependent kinase-like 5 gene.**

Variants involving the *CDKL5* gene have recently been identified as a cause of X-linked intellectual disability and epileptic encephalopathy.[1–3] The gene, located on chromosome band Xp22, encodes for the cyclin-dependent kinase-like 5 protein, which serves as an important regulator for neuronal morphogenesis. It functions to maintain normal synapse activity within the central nervous system (CNS). Downregulation of the *CDKL5* gene has been linked with abnormal neurite growth and dendritic spine structure in a mouse model.[4] Furthermore, its association with the phosphorylation of MeCP2 and DNA methyl-transferase 1 suggests a role of *CDKL5* in the epigenetic regulation of gene expression.[5,6]

Cardinal characteristics of the epilepsy associated with the CDKL5 disorder are early onset, multiple seizure types,

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Key Points

- This is the first large-scale study of ketogenic diet experience in CDKL5 disorder, a severe early onset epileptic encephalopathy
- More than half of >200 patients in the International CDKL5 Disorder Database had used the ketogenic diet at one point
- Median duration of use was 17 months
- Short-term improvement was common, but there was a lack of long-term efficacy

including mixed focal and generalized, and poor response to antiepileptic drugs (AEDs).[7,8] Seizure onset occurs at a median age of 6 weeks,[7,9] and seizure types may include infantile spasms, myoclonic seizures, tonic, and generalized tonic–clonic seizures, often with a complex semiology including multiple seizures types in one epoch.[2,8] In addition to epilepsy, phenotypic features of the CDKL5 disorder include severe neurodevelopmental deficits, cortical visual impairment, gastrointestinal symptoms, and sleep disturbances.[3,8,9]

Despite the use of intensive polypharmacy, the intractable nature of CDKL5-associated epilepsy may prompt clinicians to consider employing nonpharmacological interventions. Ketogenic diet (KD) has been proposed over the past decade as a treatment option for refractory epilepsy.[10] It was first introduced in the 1920s, based on the hypothesis that the simulation of metabolic starvation can induce antiepileptic effects via production of ketone bodies.[10] Adequate protein for growth must be provided in addition to high-fat, low-carbohydrate content to maintain ketosis within the CNS. Mouse model studies have demonstrated neuroprotective effects of the KD through the upregulation of mitochondrial uncoupling proteins leading to a decrease in seizure-related reactive oxygen species.[11,12] Other proposed antiepileptic mechanisms include the production of glutathione peroxidase and increased γ-aminobutyric acid synthesis via modification of the tricarboxylic acid cycle.[13] The most recent Cochrane review reported seizure reduction of up to 50% with KD after approximately 3 months compared to placebo in children and adolescents with refractory epilepsy.[14] Although the epilepsy characteristics of all 427 participants were not available, the two most common epilepsy syndromes represented were Lennox–Gastaut syndrome (n = 99) and infantile spasms (n = 76). However, no information was provided on which seizure types responded to the KD, and overall the quality of the evidence was poor. Nevertheless, a recent randomized control trial reported improvements in seizure frequency and severity at 4 months when KD was prescribed in addition to regular AEDs.[15]

Early commencement of KD has also been associated with a good seizure response, especially in children with infantile spasms.[16,17] This suggests potential therapeutic benefits in patients with the CDKL5 disorder, where seizure onset occurs early in infancy and includes infantile spasms in a substantial proportion.[1,2,7,8] However, the uncertainty regarding long-term efficacy and the significant side effect profile of the KD serve as major barriers against treatment maintenance.[17,18] We recently reported that, despite AED use, more than two-thirds of individuals with the CDKL5 disorder were having seizures once or more per day, emphasizing the need for further understanding of nonpharmacological management of epilepsy.[7] Previous studies have reported poor response to the diet in small samples (6 and 17 cases) with the CDKL5 disorder.[8,19] However, to date there has been no large scale study investigating the efficacy and safety profile of KD in CDKL5-associated epilepsy. Better knowledge in this area would assist clinicians in their management of CDKL5-associated epilepsy. Hence, this study sought to provide observational analyses and further insight into the role of KD for the CDKL5 disorder using parent-reported data from the International CDKL5 Disorder Database (ICDD).[9,20]

Methods

The ICDD, established in 2012, collects information from caregivers of individuals with the CDKL5 disorder through either paper or online-based questionnaires, which families can complete at their own pace, or alternatively by telephone interview.[7,9,20] The ICDD questionnaire was designed to capture comprehensive data pertaining to aspects of childhood development, phenotypic features, and natural history of the disorder. Information on epilepsy characteristics and treatment were analyzed for this study. Individuals were only included after confirmation of the pathogenicity of their *CDKL5* variant. Pathogenic variant status was classified, based on predicted functional effects, into five general groups as per described in a previous study: (1) no functional protein, (2) missense/in-frame variants within catalytic domain, (3) truncations between amino acid 172 (aa172) and aa781, (4) truncations after aa782, and (5) all other remaining variants.[7] Ethics approval was obtained from the Human Research Ethics Committee, University of Western Australia.

Baseline variables include country of residence, gender, age of ascertainment, gastrostomy status, and seizure profile including current AED use and the presence of a seizure-free period of >2 months prior to ascertainment. Families were also asked to provide information regarding history of KD treatment in their child including the age of diet commencement, perceived effects on seizures, duration of treatment, side effects including behavioral changes, and reasons for diet cessation.

Statistical analysis

Descriptive analyses were used to summarize characteristics of individuals in the study. Two-sided Fisher's exact test

was used to assess the independence of proportions. Kaplan–Meier time-to-event analysis was performed to assess the estimated probability of commencement and discontinuation of the KD over time. For analysis of time to commencement of KD, observation started at the age of seizure onset and finished at the age of commencement or age at ascertainment if the KD had not been used. For analysis of time to discontinuation of KD, the start time was the age of commencement and the finish time was the age of discontinuation or age at ascertainment if the KD was still currently being used. Log-rank tests were used for comparison of failure function between variant groups. Cox proportional hazards regression was used to estimate the hazard ratio (HR) of cessation of KD by variant group after adjusting for gastrostomy insertion (a time-varying variable). Linear regression was used to examine the linear relationship between the logarithmic transformed duration of KD use and gastrostomy and perceived change in seizure activity and/or behavior, adjusting for gender, variant group, and start age of KD. Duration of KD use was log-transformed after inspection of the residual plot. The ratio of its geometric mean (i.e., the difference among the arithmetic means on the log scale) was computed in the analysis. Negative binomial regression was used to model the linear relationships between current seizure rate (expressed as count data) and current KD use, adjusting for gender, variant group, presence of seizure-free period, and duration of epilepsy. Both crude and adjusted estimates were reported. Statistical analyses were performed using Stata (version 14.2).

Clinical vignettes were provided from patients enrolled in the CDKL5 Center of Excellence clinic study through Boston Children's Hospital. Families provided written informed consent for participation in this study, which was approved by the institutional review board.

Results

Baseline–Pathogenic variant status, gender, age of ascertainment

A total of 225 individuals with a confirmed pathogenic or likely pathogenic *CDKL5* variant and epilepsy, on whom some or all of the questionnaire had been completed as of November 16, 2016, were identified in the study. Among them, families of 204 individuals had responded to questions in relation to KD.

The median age of ascertainment was 4.8 years (n = 204, range = 0.3–33.9 years), with a median age of seizure onset of 6 weeks (range = 1 day–65 weeks). More than half (52.0%, n = 106) of the families were from the U.S.A., followed by United Kingdom (9.3%, n = 19), Australia (6.9%, n = 14), and Germany (6.4%, n = 13). Previous or current use of KD was reported for approximately half of the cases (51.0%, n = 104), with a higher percentage (59.4%) of use for the U.S.A. compared with other countries (41.8%; Fisher's exact test, p = 0.017; Table 1). Males were also more likely to have used the KD than females (Fisher's exact test, p = 0.013). About one-third of individuals with previous or current use of KD had a history of gastrostomy (36.5%, n = 38). Among them, 10 of 30 (33%) and 20 of 30 (66%) were known to have had the procedure performed prior to or after commencement of KD, respectively. The variant group distribution is summarized in Table 1. There was no difference in variant group for individuals with or without previous/current use of KD (Fisher's exact test, p = 0.889).

Commencement of KD and duration of use

Among those with previous or current use of KD (n = 104), commencement age data were available for 98 cases (94.2%). Time-to-event analysis showed that the median time to commencement of KD after seizure onset was 4 years (95% confidence interval [CI] = 3–6) during 788.6 person-years of follow-up (Fig. 1). No apparent difference in commencement time by variant group was noted (log-rank test, $\chi^2[4] = 1.96$, p = 0.743).

Using time-to-event analysis, the median duration of diet use was 17 months (95% CI = 9–24) during the 159.1 person-years of observation (Fig. 2), or on average 1.8 years of observation for the 87 individuals with data on KD duration. Using Cox regression, individuals with missense/in-frame variants within the catalytic domain tended to be on the diet for a longer period than those with no functional protein, before and after accounting for the effect of gastrostomy (unadjusted HR = 0.59, 95% CI = 0.30–1.19, p = 0.140, adjusted HR = 0.61, 95% CI = 0.30–1.23, p = 0.168).

Of those who had started the KD, 33 of 103 (32.0%) remained on it at the time of ascertainment. Data on the duration of KD use were available for 87 individuals. Individuals who had had gastrostomy after commencement of the KD had a slightly longer duration of use compared to those who never had gastrostomy, after accounting for gender, variant group, and start age of KD (geometric mean ratio = 1.66, 95% CI = 0.78–3.56, p = 0.185). In the same model, increase in age at commencement of KD appeared to be positively associated with the duration of KD use (geometric mean ratio = 1.06, 95% CI = 0.96–1.18, p = 0.253). Data on KD fat to carbohydrate and protein ratio were available for 35 cases (36.4%). The most common ratio was 3:1 (n = 13, 37.1%), followed by 4:1 (n = 11, 31.4%) and <3:1 (n = 6, 17.1%).

Effects on seizures

Changes in seizure activity (both positive and negative) after commencement of the KD were reported by the caregivers of two-thirds of individuals (66.4%, 69 of 104). Positive effects on seizure characteristics were described in 88% (61 of 69) of these cases, equivalent to more than half (61 of 104, 58.7%) of those commencing the diet, whereas worsening of seizure activity was reported for the remaining 12% (eight of 69), equivalent to 8% (eight of 104) of the total.

Table 1. Characteristics of the study population

	KD use	No KD use	Total
Number of individuals (%)	104 (51.0)	100 (49.0)	204 (100.0)
Age of ascertainment,[a] n (%)			
<1.5 years	5 (15.6)	27 (84.4)	32 (100.0)
>1.5–6 years	51 (53.1)	45 (46.9)	96 (100.0)
7–12 years	30 (65.2)	16 (34.8)	46 (100.0)
>12 years	18 (60.0)	12 (40.0)	30 (100.0)
Country of residence,[b] n (%)			
United States of America	63 (59.4)	43 (40.6)	106 (100.0)
United Kingdom	8 (42.1)	11 (57.9)	19 (100.0)
Australia	7 (50.0)	7 (50.0)	14 (100.0)
Germany	7 (53.8)	6 (46.2)	13 (100.0)
Canada	2 (28.6)	5 (71.4)	7 (100.0)
France	2 (33.3)	4 (66.7)	6 (100.0)
Others[c]	15 (38.5)	24 (61.5)	39 (100.0)
Sex,[d] n (%)			
Female	84 (47.4)	93 (52.5)	177 (100.0)
Male	20 (74.1)	7 (25.9)	27 (100.0)
Mutation status,[e] n (%)			
No functional protein	27 (46.6)	31 (53.4)	58 (100.0)
Missense/in-frame mutations	33 (55.9)	26 (44.1)	59 (100.0)
Truncations between aa172 and aa781	29 (50.9)	28 (49.1)	57 (100.0)
Truncations after aa782	9 (47.4)	10 (52.6)	19 (100.0)
Mutation not grouped	6 (54.5)	5 (45.5)	11 (100.0)
Median current seizure frequency (min,max), episodes per day	3 (0,20)[f]	1 (0,70)[g]	2 (0,70)[h]

Probability was determined by two-sided Fisher's exact test of independence of proportions.
aa, amino acid; KD, ketogenic diet.
[a] $p < 0.001$.
[b] $p = 0.017$ (U.S. vs. non-U.S.).
[c] Argentina, Belgium, Bermuda, Brazil, Bulgaria, Chile, China, Denmark, Finland, Hungary, India, Indonesia, Israel, Italy, Korea, Luxembourg, New Zealand, Norway, Poland, Portugal, Romania, Russia, Saudi Arabia, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, The Netherlands, and Turkey.
[d] $p = 0.013$
[e] $p = 0.889$.
[f] $n = 99$.
[g] $n = 90$.
[h] $n = 189$.

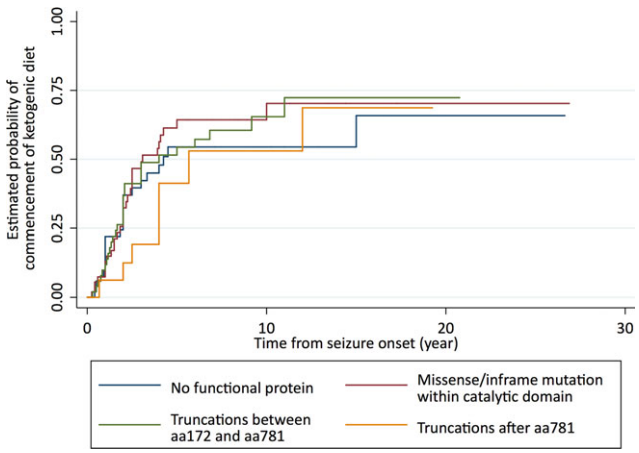

Figure 1.
Kaplan–Meier estimate of failure function for commencing ketogenic diet since seizure onset.
Epilepsia © ILAE

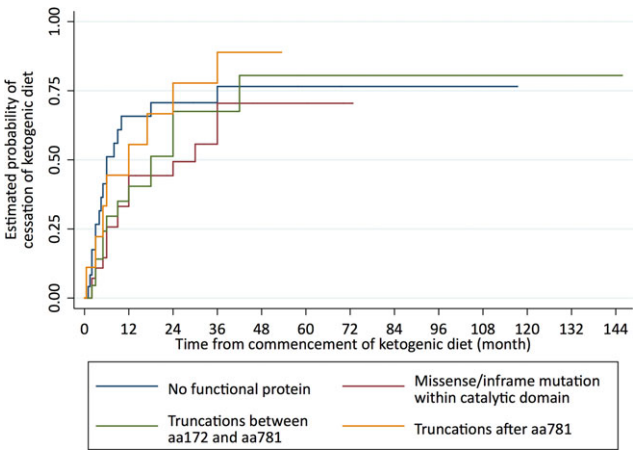

Figure 2.
Kaplan–Meier estimate of failure function for the duration of treatment with ketogenic diet.
Epilepsia © ILAE

Positive effects were reported for 50 of 57 (88%) females and 11 of 12 (92%) males, and the mean age at commencement was 2.8 years (standard deviation [SD] = 2.8) in those for whom positive effects were reported and 1.7 years (SD = 0.7) for those with reported negative effects. There was no association between positive and negative reports and

mutation group. Duration of KD use was more than twice as high (geometric mean ratio = 2.19, 95% CI = 0.72–6.72, p = 0.166) in the positive group compared to the negative one. Of the 61 individuals with positive effects, 80% (n = 49) reported improvement in seizure frequency, 51% (n = 31) in seizure duration, and 61% (n = 37) in seizure intensity.

The median current seizure frequency at time of ascertainment was 1 (range = 0–70, n = 90) episode per day among individuals who had never been on the KD, 2 (range = 0–10, n = 32) for those currently on the KD, and 3 (range = 0–20, n = 67) for those who had ceased using the KD. Thus, compared to those who had never been on the KD, the seizure rates were higher in those whose KD regime had been ceased (incidence rate ratio [IRR] = 1.74, 95% CI = 1.20–2.53) and slightly higher in those currently on the diet (IRR = 1.20, 95% CI = 0.75–1.93) after adjusting for gender, variant group, presence of seizure-free period, and duration of epilepsy. The median number of AEDs currently used was 2 (range = 0–6, n = 200). No difference in usage was observed among those who had never been on KD, were currently on KD, and had ceased KD use (Kruskal–Wallis rank test, $\chi^2[2] = 0.519$, p = 0.772).

Effects on behaviors

Behavioral changes were reported for 39 (37.5%) of 104 individuals. Improvement in alertness was the most common descriptor among the positive changes reported (n = 19) in those with improvements in seizure control. Other reported changes included increased eye contact (n = 4) and smiling (n = 3). Decline in motor and social interaction skills was reported for six, 5.8% of those who tried the KD, and of these, three parents had also mentioned the KD when asked about serious medical concerns occurring at the time of regression. A further four also attributed some sort of regressive episode to the KD but had not specifically commented about behavioral change. Thus, of those who had used the KD, a maximum of 10 (9.6%) could have potentially regressed in association with the KD. Overall, parents reported that their child had had a period of regression in 99 of 204 (48.5%) of cases in this study.

Cessation of KD and side effects

The most common reason cited for discontinuing the KD was the lack of long-term efficacy (51%, 36 of 70). Other reasons include severe side effects (n = 18, 26%), worsening seizure activity (n = 5, 7%), and difficulty in diet administration (n = 3, 4%). Two or more concurrent reasons were reported in three individuals who ceased the diet. There was one individual who reported ceasing the diet secondary to the detection of a contraindicating metabolic disorder.

Among individuals in whom some positive effects on seizures were reported but who had not continued the KD (57%, 35 of 61), the reported reasons for ceasing were as follows: (1) no improvement or recurrence of seizures (n = 18, 51%); (2) severe side effects (n = 7, 20%) including gastroesophageal reflux disease, gastroenteritis, renal dysfunction, liver dysfunction, and osteoporosis; (3) difficulty in administering diet (n = 3, 9%); (4) lack of tolerance (n = 2, 6%); and (5) insufficient weight gain (n = 1, 3%).

Inclusive of those who did not tolerate the diet (n = 18), one-third of individuals (31.7%, 33 of 104) in the study experienced one or more side effects after commencing the KD. Gastrointestinal side effects such as nausea, vomiting, and constipation were most commonly observed, at 15.4% (16 of 104). Inadequate weight gain or significant weight loss secondary to poor dietary intake were reported in eight (7.7%) individuals. Other side effects include ketoacidosis (n = 2), hepatotoxicity (n = 2), renal dysfunction including nephrolithiasis (n = 2), sinus tachycardia (n = 1), and osteoporosis (n = 1). There were no reported cases of mortality associated with KD.

Vignettes

Two case examples are provided from the Boston Children's Hospital CDKL5 Center of Excellence, one a gastrostomy-tube–fed toddler and one an orally fed infant (Boxes 1 and 2). Both had positive benefits, but there were also side effects in each case and waning efficacy over time, although there was possibly improved long-term seizure control. The second patient was included in the ICDD.

Box 1. Clinical Vignette Case 1

A 6-year-old patient with CDKL5 disorder (missense variant c.637G>C, p.Gly213Arg) had seizure onset at 11 weeks including focal seizures, myoclonic seizures, tonic seizures, and tonic–clonic seizures.[40] She had late onset of spasms at 15 months. Ketogenic diet was started at 2 years, 11 months by gastrostomy tube and later G-J tube. She had up to 10 seizures per day, including tonic, generalized tonic–clonic, and clusters of spasms. She tolerated the diet well and had an initial approximately 80% reduction in seizure frequency, then near resolution of seizures with only rare questionable tonic events for approximately 8 months on a ratio of 2.75:1 with moderate ketosis. She also had improvement in alertness and interaction. Gradually tonic and tonic–clonic seizures recurred. Duration of treatment was 1 year, 9 months. Diet was stopped during a hospitalization for aspiration pneumonia due to hypoglycemia and acidosis in the setting of illness. She had difficulty with gastrointestinal dysmotility, vomiting, poor weight gain, and frequent hospital admissions. Her medical status improved after discontinuation of ketogenic diet, and seizures remained stable.

Box 2 . Clinical Vignette Case 2

A now 15-year-old female with CDKL5 disorder (truncating variant c.1791delC, p.Tyr598Thrfs*18) had seizure onset at 13 weeks with generalized tonic–clonic seizures. Over time, she had daily seizures including brief tonic seizures in clusters, spasms, myoclonic seizures, hypermotor seizures, and focal seizures with an abnormal smile. She initiated oral ketogenic diet at 7 months of age, and had initially an approximately 50% reduction in seizure frequency. Tonic and myoclonic seizures resolved after 5 months of treatment, with residual more subtle focal seizures with unusual smile. She had lethargy with diet initiation that resolved within days, followed by improved interactions and responsiveness. Tonic and myoclonic seizures recurred, increasing to daily at 7 months into treatment, despite increase in ratio from 3:1 up to 4:1 with good ketosis. Diet was weaned after 8 months of treatment due to waning efficacy. Side effects of the diet included hyperuricemia and hypercalcemia, which resolved on discontinuation. During treatment, she could be weaned off all antiseizure medications. On discontinuing ketogenic diet, she achieved excellent sustained seizure control on lamotrigine monotherapy, with >2-year periods of seizure freedom.

Discussion

Individuals with the CDKL5 disorder suffer from early onset of epilepsy, which remains poorly controlled despite use of multiple AEDs. KD has been proposed as a second- or third-line intervention for pediatric refractory epilepsy of different etiologies. Our study represents the first large-scale study to investigate the role of KD in CDKL5 disorder. We found that KD had been commonly prescribed to manage CDKL5-associated epilepsy, with reports of previous or current treatment in approximately half of the patients in the ICDD. Our findings also illustrated some benefits, even if short-term, from introducing the KD to improve seizure activity, as reported by more than half of the caregivers. Considering that our median age at commencement of diet was 4 years after seizure onset and only a small number of children (n = 5) were treated early (age < 1.5 years), there could be opportunity for an earlier treatment uptake. Additional benefits in the efficacy and safety of KD have been observed when used early in the course of the disease.[10,16] In addition, the International Ketogenic Diet Study Group recommends early use of KD in certain epilepsy disorders such as infantile spasms and Rett syndrome,[17,21] which has many similarities with the CDKL5 disorder[3,9] despite a much later age at seizure onset. Further studies are still required to confirm the hypothesis that there could be particular benefits from early KD use in this disorder. The excellent seizure outcome for the patient in the second vignette, treated in infancy, supports this hypothesis.

Contrary to findings from a previous study of epilepsy management, including the use of the KD, in CDKL5 disorder,[19] initial improvement in seizure activity was reported in more than half (61 of 104, 58.7%) of our cohort. Decrease in seizure frequency was the most commonly reported positive response. This is in accordance with results from previous trials that showed initial decrease in seizure frequency in nearly 50% of children with refractory epilepsy.[16,22–25] However, poor long-term efficacy of the KD remains as a significant problem, well documented in clinical trials, cohort studies, and systematic reviews.[10,14,22] Our findings show that more than half of individuals with CDKL5-associated epilepsy ceased the diet for this very reason. The median duration of diet of 1.4 years in our study suggests that resistance to KD is typically observed after 1 year. However, those with missense/in-frame variants within the catalytic domain tended to be on the diet for a longer period than those with no functional protein, even after accounting for the effect of gastrostomy. We had previously also found that the seizure rate overall was lower in this group.[7] Therefore, it is plausible that a beneficial effect provided by the KD was resulting in its longer duration of use. Based on our observations regarding duration of use, KD may be an appropriate short-term treatment for the management of CDKL5-associated epilepsy. Nevertheless, and consistent with some other reports in the literature,[22,26] worsening of seizures was also experienced by a small number of cases in our study. This, in addition to the single case of contraindicating metabolic disorder, suggests that KD may not be suitable for everyone with the CDKL5 disorder. Further longitudinal monitoring is required to determine adequacy and ideal duration of the KD for optimal seizure management.

Complexity in the preparation of KD meals has been reported as a major obstacle for treatment maintenance. Previous studies have recommended using an all-liquid KD to facilitate compliance, ensure sufficient ketosis, and minimize certain side effects such as acidosis and dehydration.[27,28] Greater rates of seizure response have also been reported in formula feeding as compared to solid meals (59% vs. 27%, at 12 months of treatment).[28] Our findings suggest some advantages in liquid-based KD, as those who received gastrostomy feeding maintained the diet for a longer duration, although there still may be benefit in appropriately selected orally fed children. More than a quarter of individuals with the CDKL5 disorder will require gastrostomy feeding secondary to poor oral intake.[3,9]

Behavioral improvements in areas such as attention and social functioning have been reported as additional therapeutic effects of the KD. Previous human and mouse studies on autism spectrum disorder have described positive effects of KD in aspects of cognition and social functioning.[29–31] These improvements have also been reported in prospective

studies of children with refractory epilepsy.[32,33] Our findings suggest some similar benefits in the CDKL5 disorder, with reports of improved alertness and/or social interaction (smiling, eye contact) after starting on the diet in about one-third. Similarities in neurodevelopmental features between the CDKL5 disorder and low-functioning autism suggest that the KD may also provide possible benefits for the management of CDKL5-associated behavioral abnormalities.[1,2] Future studies investigating the behavioral changes from ketosis may provide novel information to confirm this hypothesis. Conversely, either deterioration in developmental skills and behavior or a regression, which might be associated with implementation of the KD, was reported for 10 individuals (9.6%) in our study. No such developmental regression associated with the KD has been previously reported. Therefore, we had assumed that these observations were most likely associated with the natural disease progression in the CDKL5 disorder rather than a consequence of the diet. However, in the total 204 individuals in this study, a period of regression had occurred at some time in a higher proportion (~48% of cases) than the previously published 30%.[3] Negative effects on mood and psychosocial adjustments associated with the KD have also been reported in one small prospective study.[34] Moreover, a mouse model study has also demonstrated significant impairment in memory and visual–spatial learning associated with KD when compared to placebo.[35] More evidence is required to determine whether the loss of developmental skills is a potential side effect of KD use in the CDKL5 disorder.

Our study implies that the KD results in similar side effects for individuals with CDKL5 disorder as already reported in previous controlled trials and systematic reviews. Gastrointestinal problems were the most common side effect, at a rate of 15.4%, lower than the results of between 20% and 46% from the recent Cochrane review.[14] This is perhaps surprising, as we already know that the prevalence of gastrointestinal symptoms in individuals with the CDKL5 disorder is high, and therefore one might have expected a higher rather than lower rate of KD intolerance.[9] However, nearly one-fifth of our study population (17.3%) was still unable to tolerate the diet; a higher proportion than the 6–12% in previous studies.[24,36] Renal stones, estimated to occur at an average rate of 5–8%, were reported in only two individuals (<1%) from our cohort.[37] This could be accounted for by the older commencement age of KD (median = 4 years) observed in our study; diet initiation at a young age has been previously identified as a risk factor for renal calculi.[37] Metabolic acidosis, an early and usually transient side effect, was reported in two cases resulting in early diet discontinuation. Poor growth, with or without weight loss, was reported at 7.7% in our cohort. It remains as a significant cause for diet discontinuation in the pediatric population.[14] For this reason, routine monitoring of growth parameters should be performed during treatment. Deaths during KD, mentioned in previous studies, were usually secondary to severe infections and nutritional deficiencies.[23,38] We did not identify any mortality in our cases of CDKL5-associated epilepsy in the present study.

We understand the limitations of an observational approach in this study, and acknowledge the advantage of prospective or randomized controlled studies to provide better evidence regarding the efficacy of KD in the CDKL5 disorder. In addition, we acknowledge that KD is more likely to be introduced in patients who are more severely affected by refractory epilepsy. We previously reported that seizure rates tended to be higher in males and in those in the mutation group with no functional protein.[7] They were lower in those who were ambulant and in those with better communication such as the use of words or signing. Unfortunately, the current study did not have the information to allow us to measure seizure frequency prior to the initiation of KD and to confirm our hypothesis that it is being used more frequently in those with the most refractory epilepsy.

Our results may also be affected by potential confounders such as different combinations of baseline AEDs used within the study population. Treatment during the "honeymoon" phase of CDKL5-associated epilepsy could also overestimate the true efficacy of KD.[12,39] Parents were encouraged to review their medical records before answering the questionnaire to limit associated recall error. We also acknowledge that missing data from some incomplete questionnaires have resulted in varying denominators for different outcomes in this study. Lastly, our inclusion criteria of a confirmed pathogenic *CDKL5* variant status may impose a certain degree of selection bias due to the increased availability of genetic testing services only within developed countries.

Despite its limitations, the present study provides the first overview of the role of KD in CDKL5-associated epilepsy. In view of its side effect profile, KD should be commenced only under the guidance of a pediatric neurologist and specialist dietician. Strict adherence is required after initiation of the diet to achieve maximum therapeutic effect and efficacy. Future prospective trials will provide standardized assessment of the therapeutic potential and safety profile of KD in CDKL5-associated epilepsy. More evidence is needed to determine whether it is suitable as a first-line agent for seizure control. Longitudinal analysis will also be required to guide clinical decisions and determine the long-term outcomes for those who remained on the diet.

In conclusion, this study demonstrates better response to KD than has been reported for antiseizure medications in CDKL5 disorder, with a tolerance and side effect profile similar to that of a broader epilepsy population. Thus, we would suggest early consideration of use of KD in this population, while also recognizing that the effect may not be sustained in the long term. Further data will be needed to determine whether there is any disease-modifying effect of early use of KD compared to later use.

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Disclosure

The authors declare no conflicts of interest. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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