Manuscript Details

Manuscript number	EPIRES_2018_240_R1
Title	Vagus nerve stimulation for the treatment of refractory epilepsy in the CDKL5 Deficiency Disorder
Article type	Research Paper

Abstract

Background: Variants within the CDKL5 gene result in a severe epileptic encephalopathy now known as the CDKL5 Deficiency Disorder. Phenotypic characteristics include global developmental delay and early seizure onset with poor response to anti-epileptic medications. Vagus nerve stimulation (VNS) has been used in other populations as an adjunct treatment for refractory epilepsy with seizure reduction reported in over half of patients. This study aimed to investigate the role of VNS in the CDKL5 Deficiency Disorder. Methods: The International CDKL5 Disorder Database collects information on individuals with the CDKL5 Deficiency Disorder. Families provide information regarding seizure characteristics and their pharmaceutical and non-pharmaceutical management including VNS use. Descriptive statistics and time to event analyses were performed. Clinical vignettes were also provided from patients attending the CDKL5 Center of Excellence at Children's Hospital Colorado. Results: Individuals who had a pathogenic CDKL5 variant and on whom information regarding VNS treatment was available were identified (n=222). Previous or current use of VNS was reported for 38 (17.1%), with a median age at implantation of 4.9 years. Improvements in seizure control were reported in over two-thirds (25/36, 69%); including reduction in frequency (17/25, 68%), duration (18/25, 72%) and intensity (15/25, 60%) of seizures. Median duration of VNS use before any seizure improvement was 73 days. Behavioural changes such as improved mood and alertness were reported in nine individuals. Early termination of VNS secondary to side effects was reported in three cases. There was no reduction in number of AEDs for those with VNS treatment. Conclusion: Our study suggests that VNS is a generally safe and effective adjunct treatment for CDKL5-associated epilepsy. Additional benefits such as mood and behavioural improvements provide further support of its use in the CDKL5 Deficiency Disorder. Future studies are required to determine the optimal settings and therapeutic potential for this treatment.

Keywords	Vagus Nerve Stimulation; CDKL5 Deficiency Disorder; Cyclin-dependent kinase-like 5 gene; Refractory epilepsy
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Study Highlights.docx [Highlights]

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Research Data Related to this Submission

There are no linked research data sets for this submission. The following reason is given:
The data that has been used is confidential

Professor Treiman
Editors-in-Chief
Epilepsy Research

Dear Professor Treiman

RE: Vagus nerve stimulation for the treatment of refractory epilepsy in the CDKL5 Deficiency Disorder.

We would appreciate if you would consider for publication in your journal this article presenting our original research about the use of vagus nerve stimulation in the management of refractory epilepsy in in the CDKL5 Deficiency Disorder, a rare epileptic encephalopathy.

Using the International CDKL5 Disorder Database as a data source, we sought to determine the effects of vagus nerve stimulation on individuals with the CDKL5 Deficiency Disorder who suffer from debilitating epilepsy, and investigate the impact from a caregiver perspective. We believe that caregiver viewpoints are crucial in determining the benefits of this approach to seizure management in this population. We believe that our findings will improve the knowledge base regarding vagus nerve stimulation in CDKL5-associated epilepsy.

All authors claim no conflict of interest or any financial interest. This study is supported by the International Foundation for CDKL5 Research. All authors have read the manuscript and agreed to it being submitted for publication. All individuals listed as authors meet the appropriate authorship criteria, nobody who qualifies for authorship has been omitted from the list of authors, and contributors and funding sources have been properly acknowledged. Funding bodies for this work had no involvement in the study design, data collection and analysis, or manuscript preparation.

Our submitted work is our own, no copyright was breached in seeking its publication, and it has not been published and nor is being considered for publication elsewhere. As the corresponding author, my contact details are below. We look forward to hearing from you.

Best wishes
Associate Professor Helen Leonard

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Professor Treiman
Editor-in-Chief
Epilepsy Research

Dear Professor Treiman,

RE: Vagus nerve stimulation for the treatment of refractory epilepsy in the CDKL5 Deficiency Disorder.

Thank you for your consideration of our article about the use of vagus nerve stimulation in the management of refractory epilepsy in the CDKL5 Deficiency Disorder. We greatly appreciate the reviewers for their complimentary comments and suggestions. We have reviewed the comments and thoroughly revised the manuscript.

Reviewer 1: “We encourage the authors to proceed in this line of investigation in a longitudinal and prospective manner. Only a minor observation: there is an extra "s" letter in the first paragraph of the Discussion section.”

Response: We have removed the extra s in the first paragraph of the discussion section as advised. We are looking into prospective and longitudinal studies for our future research.

Reviewer 2: “ Review the wording in 1st sentence of "Results" section ("as at 23th")” “Consider using the term "variant" instead of mutation as recommended in 2015 American College of Medical Genetics guidelines”

Response: We have change “as at 23th” to “as of 23rd” as advised. We have changed all terms of “mutations” to variants as recommended for our abstract and manuscript.

We hope you find the revised manuscript suitable for publication and look forward to hearing from you.

Best wishes
Associate Professor Helen Leonard

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HIGHLIGHTS

- This is the first study to describe the use of VNS in CDKL5 Deficiency Disorder.
- One in six of our study population of over 200 patients received VNS treatment.
- Improvement in seizure activity was reported for over two thirds of those treated.

ABSTRACT

Background: Variants within the CDKL5 gene result in a severe epileptic encephalopathy now known as the CDKL5 Deficiency Disorder. Phenotypic characteristics include global developmental delay and early seizure onset with poor response to anti-epileptic medications. Vagus nerve stimulation (VNS) has been used in other populations as an adjunct treatment for refractory epilepsy with seizure reduction reported in over half of patients. This study aimed to investigate the role of VNS in the CDKL5 Deficiency Disorder.

Methods: The International CDKL5 Disorder Database collects information on individuals with the CDKL5 Deficiency Disorder. Families provide information regarding seizure characteristics and their pharmaceutical and non-pharmaceutical management including VNS use. Descriptive statistics and time to event analyses were performed. Clinical vignettes were also provided from patients attending the CDKL5 Center of Excellence at Children's Hospital Colorado.

Results: Individuals who had a pathogenic CDKL5 variant and on whom information regarding VNS treatment was available were identified (n=222). Previous or current use of VNS was reported for 38 (17.1%), with a median age at implantation of 4.9 years. Improvements in seizure control were reported in over two-thirds (25/36, 69%); including reduction in frequency (17/25, 68%), duration (18/25, 72%) and intensity (15/25, 60%) of seizures. Median duration of VNS use before any seizure improvement was 73 days. Behavioural changes such as improved mood and alertness were reported in nine individuals. Early termination of VNS secondary to side effects was reported in three cases. There was no reduction in number of AEDs for those with VNS treatment.

Conclusion: Our study suggests that VNS is a generally safe and effective adjunct treatment for CDKL5-associated epilepsy. Additional benefits such as mood and behavioural improvements provide further support of its use in the CDKL5 Deficiency Disorder. Future studies are required to determine the optimal settings and therapeutic potential for this treatment.

Keywords: Vagus Nerve Stimulation; CDKL5 Deficiency Disorder; Cyclin-dependent kinase-like 5 gene; Refractory epilepsy

TITLE: Vagus nerve stimulation for the treatment of refractory epilepsy in the CDKL5 Deficiency Disorder.

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INTRODUCTION

Variants within the *CDKL5* gene result in a severe epileptic encephalopathy now known as the CDKL5 Deficiency Disorder.(Fehr et al., 2013; Hector et al., 2017; Tao et al., 2004) Phenotypic traits include global developmental delay leading to severe intellectual and motor impairment, refractory epilepsy, dysregulated sleep and gastrointestinal dysmotility problems.(Fehr et al., 2013; Mangatt et al., 2016) Disruption of neuronal morphogenesis and normal synaptic activity is hypothesized to be the main pathophysiological process resulting from downregulation of the *CDKL5* gene.(Chen et al., 2010; Fuchs et al., 2014) Individuals with CDKL5-associated epilepsy generally present with early onset seizures of variable types and complex semiology including infantile spasms and generalized tonic-clonic seizures.(Fehr et al., 2016; Moseley et al., 2012) These seizures respond poorly to anti-epileptic drugs (AEDs), with over two-thirds of patients having daily seizure occurrence despite multiple AEDs.(Fehr et al., 2016) The severity and refractory nature of CDKL5-associated epilepsy prompts clinicians to consider non-pharmacological treatments.

Vagus Nerve Stimulation (VNS) was first proposed in the late 18th century as a treatment for seizures pertaining to its ability to reduce heart rate and cerebral perfusion.(Lanska, 2002) It involves intermittent electrical stimulation of the vagus nerve through a pulse generator device.(Groves and Brown, 2005) Significant changes in perfusion within cerebral regions of thalamus, hypothalamus, hippocampus and amygdala were observed in patients upon left cervical VNS activation.(Henry et al., 2004) While the exact mechanism remains unknown, the antiepileptic effect may result from rapid alteration of synaptic activities within the thalamus. Other postulated hypotheses include release of cerebral noradrenaline, pharmacological changes within the solitary nucleus and modulation of the reticular activating system.(Krahl et al., 1998; McLachlan, 1993; Walker et al., 1999) VNS was approved by the

US Food and Drug Administration (FDA) in 1997 as an adjunct treatment for intractable partial seizures in adults and children aged 12 years or older.(Schachter, 2002) Treatment uptake is likely to increase with the recent US FDA approval for VNS to be used in children as young as 4 years of age. (Brooks, 2017; U.S. Food and Drug Administration, 2017) VNS has been shown to provide good outcomes in refractory epilepsy, with significant seizure reduction for over half of all adult and paediatric patients reported in studies including clinical trials.(Alexopoulos et al., 2006; Elliott et al., 2011a; Elliott et al., 2011b; Englot et al., 2011; Galbarriatu et al., 2015; Klinkenberg et al., 2012; Orosz et al., 2014; Rossignol et al., 2009) A recent meta-analysis, which investigated 74 studies including 3321 patients, reported an overall rate of seizure reduction of 51% after 12 months of VNS therapy.(Englot et al., 2011) Better seizure control was reported for paediatric patients compared to adults (55% vs 50% seizure reduction).(Englot et al., 2011) Furthermore, a higher rate of seizure reduction at a mean of 62% was reported in children younger than 6 years old.(Englot et al., 2011) This could suggest additional benefits for children with the CDKL5 Deficiency Disorder who suffer from early onset epilepsy. Optimal treatment responses were also observed in patients with post-traumatic epilepsy, idiopathic epilepsy and epileptic encephalopathy including Lennox-Gastaut syndrome.(Englot et al., 2011; Orosz et al., 2014)

Apart from its antiepileptic effect, VNS treatment has been associated with improvement in psychosocial functioning such as mood and alertness.(Elger et al., 2000; Harden et al., 2000) This could be promising for individuals with the CDKL5 Deficiency Disorder who experience severe neurodevelopmental impairment and sleep disturbances.(Fehr et al., 2013; Mangatt et al., 2016) Furthermore, retrospective studies showing good long-term seizure outcomes with VNS suggest that it may be a more preferable option compared to other non-pharmacological treatments such as the ketogenic diet.(Lim et al., 2017; Vaccarezza and Silva, 2015). Better

seizure control in individuals with the CDKL5 Deficiency Disorder has also been associated with higher functional abilities such as walking and verbal communication.(Fehr et al., 2016) To date, there has been no study which investigated the efficacy of VNS in CDKL5-associated epilepsy. Hence, our study aims to provide an observation analysis and additional insight into VNS use in the CDKL5 Deficiency Disorder using data from the International CDKL5 Disorder database (ICDD).

MATERIALS AND METHODS

The International CDKL5 Disorder Database (ICDD), which was established in 2012, collects information from caregivers of affected individuals through online-based and paper-based questionnaires as well as phone interviews. The purpose of the registry is to capture comprehensive data pertaining to aspects of childhood development, phenotypic characteristics and natural history of the CDKL5 Deficiency Disorder. Information on epilepsy characteristics and treatment were analysed 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: i) no functional protein, ii) missense/in-frame variants within catalytic domain, iii) truncations between aa172 and aa781, iv) truncations after aa782 and v) all other remaining variants.(Fehr et al., 2015) Ethics approval was obtained from the Human Research Ethics Committee, University of Western Australia.

Baseline variables included country of residence, gender, age of ascertainment, gastrostomy status and seizure profile. Families were also asked to provide information regarding their child's seizure management including AED and non-pharmaceutical treatments. To describe

VNS usage, parents reported the age of VNS implantation, perceived effects on seizures, side effects including behavioural changes, and (where appropriate), reasons for its cessation.

Descriptive analyses were used to summarise the characteristics of individuals in the study. Fisher's exact test of independence was used to assess differences in characteristics between the VNS users and non-users. Kaplan Meier time-to-event analysis was performed to assess the time to implantation of the VNS. For this analysis observation started at the age of seizure onset and was censored at the age of implantation of the VNS or age at ascertainment if the VNS had not been used. The Log-rank test was used to test for equality of survival functions among different variant groups. Negative binomial regression was used to model the linear relationships between current seizure rate (expressed as count data) and current VNS use, adjusting for gender, variant group, presence of seizure free period and duration of epilepsy. The Kruskal-Wallis rank test was used to evaluate differences in number of AEDs used among VNS non-users, current and past users. Statistical analyses were performed using Stata (version 14.0).

Clinical vignettes were provided from patients attending the CDKL5 Center of Excellence at Children's Hospital Colorado with approval by the institutional review board.

RESULTS

A total of 222 individuals with a confirmed pathogenic or likely pathogenic *CDKL5* variant and epilepsy, for whom their families had responded to questions in relation to VNS as of 23rd March 2018, were identified. The median age at ascertainment was 4.6 years (range 0.3-33.9 years) with a median age at seizure onset of 6 weeks (range: 1 day – 104 weeks). Characteristics of the study population by VNS usage are summarized in Table 1. Half (50.4%) of the families

were from the US followed by United Kingdom (8.1%), Australia (6.3%) and Germany (5.9%) (Table 1). Previous or current use of VNS was reported for 17.1% (n=38) of these cases. There was a higher percentage of use for the UK (33.3%), US (22.3%), and Australia (21.4%) compared with other countries (5.1%) (P=0.004). Males and females were equally likely to have had a VNS inserted (16.7% and 17.2%, respectively; P = 0.591). There was no difference in variant group for individuals with or without previous/current use of VNS (P = 0.631).

Commencement of VNS

Among those with previous or current use of VNS (n=38), commencement age data were available for 36 cases (94.7%). For these 36 cases, the median age at implantation of VNS was 4.9 years (range 1.3-20 years). Time-to-event analysis showed that the probability of VNS commencement after seizure onset was 25% at 10 years (95% confidence interval [CI] 6,17 years) during 1,255 person-years of follow-up. No apparent difference in commencement time by variant group was noted (P = 0.456) (Fig. 1).

Effects on Seizures

Of the 38 cases with a history of VNS use, information on the effects of VNS use on seizures was provided for 36. Improvements in seizure activity after implantation of VNS were reported for slightly more than two thirds of individuals (25/36, 69%). These improvements included reductions in the duration (18/25, 72%), frequency (17/25, 68%,), and intensity (15/25, 60%,) of seizures. A reduction in post-ictal recovery time following seizure activity was reported for one individual. The median duration of VNS use before any seizure improvement was 73 days (range: 1 day – 24 months, n=24). No cases of complete seizure freedom were reported following VNS implantation.

The median current seizure frequency was 1.7 (range 0-70, n=170) episodes per day among individuals who never used the VNS, 3 (range 0-12, n=32) for those currently using the VNS and 2.6 (range 1-15, n=5) for those who had ceased using the VNS. Compared to those who never used the VNS, the seizure rates appeared similar in those currently using the VNS (IRR 1.28, 95% CI 0.74,2.22) and in those who had ceased using the VNS (IRR 1.76, 95% CI 0.62,5.00), after adjusting for gender, variant group, presence of seizure free period and duration of epilepsy.

AEDs and other treatment

The median number of AEDs being used among those current and past VNS users was 3 (range 0-6, n=38). No difference in usage was observed among those who never used the VNS, were currently using the VNS or had ceased using the VNS ($P = 0.304$). The majority of individuals (87%, 33/38) who received VNS therapy were reported to have previous or current use of the ketogenic diet for seizure control.

Behavioural changes

Behavioural changes were noted in nine individuals upon VNS implantation (9/38, 24%). In seven individuals (19%, 7/36) an increase in alertness following implantation of the VNS was reported and in three (8%, 3/36) there was an apparent improvement in mood.

Side effects

Adverse effects post VNS implantation were noted in 13% of cases (n= 5). Deterioration in behaviour, eating or sleep quality was reported in three cases (8%, 3/36). The VNS had been switched off in two of these cases secondary to eating problems or worsening sleep apnoea. ICU admission and ventilation support were required in a third individual secondary to severe

vocal cord palsy. In two other individuals, the VNS had also been turned off due to poor efficacy (one related to overall seizure control).

Clinical Vignettes

The following clinical vignettes describe children who received VNS therapy at the young age of one and two years respectively. Both cases reported delayed effects but overall benefits upon VNS implantation.

Case 1:

This 4-year-old girl with a Y262H missense variant in the kinase domain of the *CDKL5* gene had onset of seizures at 2 weeks of age characterized by bilateral stiffening followed by a cluster of bilateral jerks. She went on to have persistent tonic seizures and epileptic spasms without evidence of hypsarrhythmia. Several EEG assessments have captured frequent focal electrographic seizures without clinical correlate that have been refractory to treatment. She has had trials of nine anti-seizure medications with no more than minimal benefit. Ketogenic diet was initiated at 4 months of age and weaned at 14 months due to lack of efficacy. VNS was placed at 1 year of age and was not thought to be effective, however at 3 years of age she had the VNS turned off for one day for a surgical procedure which resulted in significant worsening of seizures and status epilepticus until the VNS was turned back on. The family, now feels that the VNS may be helping given that event, although the seizure frequency (several per day) is the same as prior to the surgery.

Case 2:

This 7-year-old girl with a deletion of exon 10 and 11 of the *CDKL5* gene originally presented at 6 weeks of age with infantile spasms with hypsarrhythmia. She initially responded to ACTH but epileptic spasms returned later in infancy and ACTH did not prove helpful on repeat trial.

She had frequent clusters of seizures; mostly hypermotor, tonic spasms sequence seizures until 2.5 years of age. She now has more sporadic tonic seizures and epileptic spasms several times per day. She has tried 11 anti-seizure medications and was on the ketogenic diet for two years without clear improvement. VNS was placed at 2 years of age and did not appear to be helpful initially but seizures have decreased modestly since then. It is unclear if these are related. The family notes that swiping the magnet on the VNS can be helpful in stopping a cluster of seizures.

DISCUSSION

Our study represents the first ever large-scale study to investigate the role of VNS in the management of CDKL5-associated epilepsy. We found that only a small proportion of individuals (17.1%) from the ICDD have received VNS treatment but of these over two thirds experienced clinically important improvements in seizure activity following VNS implantation. Shorter duration and reduction in frequency and intensity of seizures were most commonly described, although there were no reports of complete seizure freedom. We did not identify any relationship with genotype. Our findings are generally concordant with results from a recent meta-analysis, showing seizure reduction in over two-thirds (74.6%) but complete seizure freedom in only a very small percentage (<5%) of patients post VNS implantation.(Englot et al., 2011) Other studies have reported relatively low rates of seizure freedom ranging from 2 – 8% .(Ben-Menachem et al., 1994; De Herdt et al., 2007; Orosz et al., 2014) Prospective, long-term studies are required to determine if complete seizure freedom can be achieved with VNS in the CDKL5 Deficiency Disorder.

In our study, we found the median age at time of implantation to be noticeably earlier compared to other retrospective studies (4.9 years vs 10 – 12 years). The age difference may be explained

by the fact that the epileptic encephalopathy associated with the CDKL5 Deficiency Disorder has a complex semiology with a typical onset around 6 weeks of age.(Fehr et al., 2016) Significant seizure reduction was reported at a median duration of 73 days post VNS insertion, and, similar to other studies, in most patients within 3 months of treatment onset.(DeGiorgio et al., 2000; Morris and Mueller, 1999; Salinsky et al., 1996; Siddiqui et al., 2010) A meta-analysis of 3321 patients has shown a significantly higher response with early VNS implantation in children younger than six years and it is possible that early treatment can also provide therapeutic benefits in CDKL5 Deficiency Disorder patients.(Englot et al., 2011) In addition, multiple studies have demonstrated a positive relationship between treatment duration and seizure control upon VNS implantation.(Alexopoulos et al., 2006; DeGiorgio et al., 2000; Orosz et al., 2014; Siddiqui et al., 2010) Our findings show that at time of ascertainment to the study the majority of individuals still had their VNS devices switched on, supporting its ongoing and cumulative effect. However similar to previous retrospective studies, our findings show that VNS is not associated with significant reduction in the number of AEDs despite better seizure control.(Alexopoulos et al., 2006; De Herdt et al., 2007; Murphy et al., 2003; Orosz et al., 2014)

Our study found a lower uptake of VNS therapy as compared to the ketogenic diet, the other non-pharmacological treatment commonly used in CDKL5-associated epilepsy. Interestingly, the majority of those who received VNS treatment in the study were reported to have previous or current use of the ketogenic diet where the median age of onset was four years slightly earlier than for VNS.(Lim et al., 2017) This suggests that VNS may be being prescribed for patients whose seizures remained poorly controlled despite dietary management. It also highlights the challenges of seizure control in the CDKL5 Deficiency Disorder which often require multiple treatment modalities. Potential synergistic effects have been described in a recent study when

VNS is used in conjunction with ketogenic diet.(Kossoff et al., 2007) Unfortunately, our study did not have sufficient information to confirm the above association. Further research is needed to assess the efficacy and safety of this combination therapy in CDKL5-associated epilepsy.

Improvement in behavioural aspects such as alertness and mood have been reported as additional benefits of VNS therapy.(Elger et al., 2000; Harden et al., 2000) Such effects were first demonstrated in initial observational studies, whereby patients with a VNS implanted reported significant improvement in mood beyond those attributed to better seizure control.(Ben-Menachem et al., 1994) Our results suggest similar effects in the CDKL5 population, with improved mood and alertness after VNS implantation reported in a small proportion. It has been hypothesized that these effects may be due to an alteration of cerebral neurotransmitter levels including serotonin, GABA and glutamate.(Ben-Menachem et al., 1994; Walker et al., 1999) Imaging studies conducted on patients receiving VNS therapy revealed decreased activity and significant hypo-perfusion in the cingulate gyrus, findings which can be associated with anti-depressant effects.(Connor Jr et al., 2012; Henry et al., 2004) Abnormal GABA and glutamate expression observed in CDKL5 murine models suggest possible advantages of VNS in tackling mood and behaviour deficits over and above its anti-epileptic effect.(Amendola et al., 2014; Sivilia et al., 2016) More evidence is required to confirm this positive association in the CDKL5 Deficiency Disorder.

Our study suggests that VNS is a comparatively well tolerated treatment for CDKL5-associated epilepsy with side effects reported in less than 10% of cases. Occurrence or worsening of breathing abnormalities after VNS implantation resulting in early treatment cessation were reported in two cases, one of whom required ventilatory support. This would be consistent with previous estimates of significant vocal cord dysfunction occurring in approximately 2% of

patients following VNS implantation.(Parhizgar et al., 2011) Respiratory distress from vocal cord palsy is likely due to effects of left vocal cord adduction from stimulation of the left recurrent laryngeal nerve during VNS therapy.(Parhizgar et al., 2011) Worsening sleep apnoea was noted in only one case from our study resulting in early treatment termination. This is in contrast to previous studies which reported increase in apnoea-hypopnoea index (AHI), a marker of severity in sleep apnoea, for the majority of patients receiving VNS therapy.(Parhizgar et al., 2011) Vocal hoarseness, a common side effect, was not reported in our study cohort.(Binnie, 2000) However, the majority of individuals with CDKL5 variants suffer from severe neurodevelopmental deficits, and have limited or no verbal communication skills.(Fehr et al., 2013) We did not identify any surgical site infection or mortality associated with VNS therapy in the present study. Longitudinal analyses will be required to determine the long-term safety profile of VNS in the CDKL5 Deficiency Disorder.

We appreciate the limitations of the observational approach necessary in the present study, and agree that a randomized control trial or a longitudinal cohort study is needed to better evaluate the efficacy of VNS in patients with the CDKL5 Deficiency Disorder. We also acknowledge that VNS therapy tended to be prescribed for patients who were especially severely affected by their epilepsy. In order to reduce associated recall bias, we encouraged parents to review records of medical history and seizure diaries prior to answering the questionnaire. Our results may be affected by missing data from some incomplete questionnaires resulting in different denominators for certain study outcomes. In addition, potential confounders such as types of baseline AEDs and VNS settings can influence treatment response even though they reflect real-world clinical situations.

CONCLUSION

Our study concludes that with appropriate surveillance VNS is a relatively safe and effective adjunct treatment to AED use for CDKL5-associated epilepsy. Furthermore, additional benefits such as mood and behavioural improvements provide further support for its use in this disorder. Future studies are required to determine the optimal settings and therapeutic potential for this treatment modality. Clinicians should consider early introduction of VNS, bearing in mind the intractable nature of CDKL5-associated epilepsy and recent FDA approval for its use in younger children.(Brooks, 2017; U.S. Food and Drug Administration, 2017)

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DISCLOSURE OF CONFLICTS OF INTEREST

None of the authors has any conflict of interest to disclose.

ETHICAL PUBLICATION STATEMENT

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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Table 1. Characteristics of the study population

	VNS use	No VNS use	Total
	n, (row %)	n, (row %)	n, (row %)
Number of individuals	38 (17.1)	184 (82.9)	222 (100.0)
Age of ascertainment, years			
< 1.5	0 (0.0)	38 (100.0)	38 (100.0)
>1.5 - 6	12 (12.0)	88 (88.0)	100 (100.0)
7 - 12	14 (26.9)	38 (73.1)	52 (100.0)
>12	12 (37.5)	20 (62.5)	32 (100.0)
Country of residence			
United States of America	25 (22.3)	87 (77.7)	112 (100.0)
United Kingdom	6 (33.3)	12 (66.7)	18 (100.0)
Australia	3 (21.4)	11 (78.6)	14 (100.0)
Germany	1 (7.7)	12 (92.3)	13 (100.0)
Canada	1 (8.3)	11 (91.7)	12 (100.0)
France	0 (0.0)	7 (100.0)	7 (100.0)
Others*	2 (4.4)	44 (95.6)	46 (100.0)
Sex			
Female	33 (17.2)	159 (82.8)	192 (100.0)
Male	5 (16.7)	25 (83.3)	30 (100.0)
Variant status			
No functional protein	9 (14.8)	52 (85.3)	61 (100.0)
Missense/in-frame variants	13 (21.0)	49 (79.0)	62 (100.0)
Truncations between	11 (17.2)	53 (82.8)	64 (100.0)
Truncations after aa782	2 (8.7)	21 (91.3)	23 (100.0)
Variant not grouped	3 (25.0)	9 (75.0)	12 (100.0)

* Others include The Netherlands, Brazil, Italy, Russia, India, New Zealand, Spain, China, Denmark, Hungary, Norway, Argentina, Belarus, Belgium, Bermuda, Bulgaria, Chile, Finland, Israel, Luxembourg, Poland, Portugal, Romania, Saudi Arabia, Slovenia, Sweden and Turkey.

Figure 1. Kaplan-Meier estimate of time to event function for commencing VNS since seizure onset, by CDKL5 variant group