Epilepsy Currents
2024, Vol. 24(3) 191-193

DOI: 10.1177/15357597241249045
journals.sagepub.com/home/epi
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Cyclin-Dependent Kinase-Like 5 Deficiency Disorder: A Calcium Channelopathy?

Epilepsy-Linked Kinase CDKL5 Phosphorylates Voltage-Gated Calcium Channel Cav2.3, Altering Inactivation Kinetics and Neuronal Excitability

Sampedro-Castañeda M, Baltussen LL, Lopes AT, Qiu Y, Sirvio L, Mihaylov SR, Claxton S, Richardson JC, Lignani G, Ultanir SK. *Nat Commun*. 2023;14(1):7830. doi:10.1038/s41467-023-43475-w

Developmental and epileptic encephalopathies (DEEs) are a group of rare childhood disorders characterized by severe epilepsy and cognitive deficits. Numerous DEE genes have been discovered thanks to advances in genomic diagnosis, yet putative molecular links between these disorders are unknown. CDKL5 deficiency disorder (CDD, DEE2), one of the most common genetic epilepsies, is caused by loss-of-function mutations in the brain enriched kinase CDKL5. To elucidate CDKL5 function, we looked for CDKL5 substrates using a SILAC-based phosphoproteomic screen. We identified the voltage-gated Ca2+ channel Cav2.3 (encoded by CACNA1E) as a physiological target of CDKL5 in mice and humans. Recombinant channel electrophysiology and interdisciplinary characterization of Cav2.3 phosphomutant mice revealed that loss of Cav2.3 phosphorylation leads to channel gain-of-function via slower inactivation and enhanced cholinergic stimulation, resulting in increased neuronal excitability. Our results thus show that CDD is partly a channelopathy. The properties of unphosphorylated Cav2.3 closely resemble those described for CACNA1E gain-of-function mutations causing DEE69, a disorder sharing clinical features with CDD. We show that these two single-gene diseases are mechanistically related and could be ameliorated with Cav2.3 inhibitors.

Commentary

Developmental and epileptic encephalopathies (DEE) are rare but devastating forms of early-onset epilepsy in which the seizures themselves contribute to detrimental effects on brain function and cognition. Almost one hundred DEE have been described and the dire nature of these epilepsies makes understanding their molecular mechanisms an urgent endeavor.[1]

Pathogenic variants in the cyclin-dependent kinase-like 5 gene (*CDKL5*) cause a DEE consisting of infantile-onset drug-resistant epilepsy, neurodevelopmental impairment, cognitive and motor deficiencies, and severe sleep impairment (CDKL5 deficiency disorder, or CDD; also known as DEE2).[2] The *CDKL5* gene is located on the X-chromosome (Xp22.13). The gene is subject to X inactivation and 80% of affected individuals are female. Affected males often exhibit poorer functional motor and communication skills.[3] While this disorder is rare (one in 40 thousand live births), it causes disproportionate disability for patients and families. The epilepsy in CDD starts early in life, in the first several weeks or months, and the seizures rapidly become resistant to treatment. Absence or dysfunction of *CDKL5*, especially missense mutations in the kinase domain, cause CDD. However, the mechanism of refractory seizures in CDD remains unclear. No specific treatment is available and conventional anti-seizure medications, and even the ketogenic diet and newer treatments such as cannabidiol, are rarely effective.[4] The neurosteroid ganaxalone, a GABA-A receptor allosteric modulator, has shown promising effectiveness for seizure control in CDD,[5] possibly by enhancing GABAergic inhibition.[6] However, understanding the molecular mechanism of neuronal hyperexcitability in greater detail could lead to a more syndrome-specific therapeutic approach.

The CDKL5 protein is a serine/threonine kinase that is enriched in brain and phosphorylates several target proteins. Its molecular underpinnings are beginning to be understood. Substrates phosphorylated by CDKL5 include a diverse range of mediators of brain development and function including those involved in cilia function, microtubule formation, cerebral organization, synaptic function, and dendritic spine structure.[2] One or more of these targets could be involved in epileptogenesis in CDD.

The current study used a phosphoproteomic screen in mice lacking the *Cdkl5* gene to search for CDKL5 target proteins.[7] The authors identified the voltage-gated calcium channel,

Cav2.3, as a phosphorylation target. Calcium channels are well-recognized modulators of cellular excitability and subcellular signaling[8] and numerous DEE are associated with mutations in the various calcium channel subtypes.[9]

In normal individuals, Cav2.3 channels are phosphorylated by CDKL5, and then pass R-type inward calcium current, participating in neurotransmitter release and the regulation of neuronal excitability. The authors created a phosphomutant Cav2.3 human construct (serine 14 to alanine) and a line of mice with the equivalent phosphomutation at the specific phosphorylation site on Cav2.3 (specifically, serine 15 in mice). In transfected HEK293 cells, electrophysiological recordings showed slower inactivation of human Cav2.3 phosphomutant channels and a greater hyperpolarizing shift in voltage-dependent activation upon cholinergic stimulation. In neurons from mice with Cav2.3 serine 15 mutation, Cav2.3 currents were also more prolonged and there was increased excitability in response to the cholinergic agonist carbachol, both of which could predispose to seizures. That is, phosphomutant Cav2.3 channels engendered hyperexcitability based on prolonged calcium currents with slower channel inactivation and increased muscarinic regulation. These findings are especially intriguing because Cav2.3 dysfunction was previously reported to cause another DEE, called DEE69, that has a phenotype similar to CDD, with intractable seizures and global cognitive impairment.[10] In DEE69, gain-of-function mutations in *CACNA1E*, the gene encoding Cav2.3, result in enhanced excitability due to slowed channel inactivation and hyperpolarized activation voltages (and hence, enhanced R-type calcium currents and hyperexcitability). These findings suggest that CDKL5, via phosphorylation of Cav2.3, normally limits calcium current and constrains excitability. The authors supported these conclusions by using induced pluripotent stem cell-derived neurons from patients with CDD. In those cells, Cav2.3 phosphorylation was diminished, lending support to the hypothesis that this channel is dysfunctional in individuals with CDD. The authors hypothesized that similar molecular mechanisms might underlie the increased seizure propensity in CDD.

CDKL5 knock-out mice exhibit behavioral impairments and EEG changes. Therefore, the authors examined their phosphomutant mice for similar behavioral and cognitive impairments and seizure susceptibility. Some deficits were documented that could correlate with human CDD, specifically, impaired learning and memory in the contextual fear conditioning task in mutants compared to wild-type mice. Among many tests of behavior, locomotion, and socialization, significant differences were seen only in home cage locomotion, wheel running, and memory formation and retention (males) and social novelty (females); both sexes displayed impaired fear conditioning. These wide-ranging behavioral observations defy ready explanation on a genotype/phenotype basis and require validation with larger animal numbers. Adult female mutant mice were slightly more sensitive to repeated low-dose kainic acid-induced seizures, but males were not. Thus, the behavioral profile and seizure sensitivity of wild-type versus mutant mice were not as robustly different as might be predicted for a model of severe epilepsy such as CDD, compared with other DEE models in which animals do display the clinical features, seizure predisposition, and profound cognitive and behavioral deficits analogous to the human disease (eg, Dravet syndrome).[11] There are several possible reasons for this discordance. First, clinical, behavioral, and electrographic features are different between mutations and between species. Second, in CDD, CDKL5 phosphorylates many target proteins besides Cav2.3. Third, while the behavioral tests chosen here span a wide range of behavioral impairments, their sensitivity and specificity might still be too low to detect differences.[12] Fourth, the repeated low-dose kainic acid protocol is but one of innumerable tests of seizure susceptibility in animal models. Future research should elucidate the role of Cav2.3 phosphorylation in behavior and learning, and clarify the sex discrepancies in Cav2.3 expression and regulation. Given that the phosphomutant mice harbor only one altered substrate/site of phosphorylation, it does not represent a definitive model of CDD, but these results do provide insight into the role of Cav2.3 on excitability and behavior and could possibly lead to a fuller understanding of CDD.

In summary, this study identified the voltage-gated calcium channel Cav2.3 as a physiological target of CDKL5-mediated phosphorylation in mice and humans, supporting this disorder as a calcium channelopathy, accounting for at least part of its pathophysiology. There is currently no available drug that inhibits Cav2.3, but the search is underway. If such a compound could be found, it would represent an advance in the effort to bring personalized medicine to a subset of severe DEE.

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Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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