# Supporting CDKL5

This is a short summary of some of the research into CDKL5 that was published in 2018. I have explained some of the background science in places but you might want to refer to some of the pages on this site (www.supporting-cdkl5.co.uk) for further information.

## The Genetics of CDKL5

**CDKL5 variants: Improving our understanding of a rare neurologic disorder.**
**Ralph Hector et al.**
**Neurology Genetics 3.6 (2017): e200.**

Not all genetic mutations are disease causing, some may cause no discernible effect and can be known as silent mutations. In this paper, the authors combined data from large-scale population sequencing studies with CDKL5 variants from new and all available clinical cohorts. They were then able to predict the pathogenicity of specific mutations using computational methods. Their analysis looked at mutations both within and outside the catalytic domain of the CDKL5 gene.
They identified 59 missense mutations in the catalytic domain, all of which were considered pathogenic as in each case the individual had been diagnosed with CDKL5. In most cases these pathogenic mutations had also been predicted by computer analysis. Outside the catalytic domain they identified 179 different missense mutations. On the whole, the authors suggest that these are not pathogenic although a number of instances are described where an apparent missense mutation is pathogenic due to other transcriptional effects. Nonsense, frameshift and splicing variants, which can occur throughout the CDKL5 gene, along with copy number variations and CDKL5 duplications are generally more difficult to categorise in terms of establishing a genotype-phenotype relationship. In particular, the authors state that more evidence is needed to establish whether there is a well defined CDKL5 duplication syndrome. Finally, they reiterate that there is substantial evidence that hCDKL5_1 is the predominant brain isoform, and that this is required for normal brain development.

## The CDKL5 Protein

**Chemical genetic identification of CDKL5 substrates reveals its role in neuronal microtubule dynamics.**
**Lucas L Baltussen et al.**
**The EMBO Journal (2018) e99763.**

Much research is focussed on identifying the substrates of CDKL5, that is, the molecules that the CDKL5 protein directly acts upon. It turns out that there may be many, suggesting the complex role CDKL5 has in brain development and function. The authors of this paper identify 3 new substrates, ARHGEF2, EB2 and MAP1S, the latter 2 of which are said to be physiological substrates within the brain. MAP1S is a protein involved in the function of microtubules which the authors show is disrupted by CDKL5 loss. Furthermore, CDKL5 appears to be a negative regulator of MAP1S, that is, MAP1S is switched off by CDKL5.


**Phosphoproteomic screening identifies physiological substrates of the CDKL5 kinase.**
**Ivan Munoz et al.**
**The EMBO journal, (2018), e99559.**

In this paper, the authors also identify 3 substrates of CDKL5. These are DLG5, CEP131 and MAP1S which was also identified in the paper above. The authors suggest that these substrates may act as important biomarkers in the diagnosis and treatment of CDKL5.

## Investigating Treatment Strategies

**CDKL5 protein substitution therapy rescues neurological phenotypes of a mouse model of CDKL5 disorder.**
**Stefania Trazzi1 et al.**
**Human molecular genetics 27.9 (2018): 1572-1592.**

This paper reports research aimed at developing protein substitution therapy for CDKL5 to compensate for the CDKL5 protein deficiency that occurs as a result of mutations in the gene. The authors report the production of a protein complex, TAT-CDKL5 , that was efficiently transduced into nerve cells using a virus carrier. They firstly, established that TAT-CDKL5 introduced into neurons maintained its physiological activity, as seen in wild-type (normal control) neurons.
They then studied the effects of protein therapy on Cdkl5 knockout mice (no Cdkl5 gene). In these studies the CDKL5 protein complex was infused directly into the brain. The authors studied the effects of protein replacement on learning, which is severely impaired in the KO mice, as well as sleep apnoea and hind-limb clasping which are characteristic in KO mice. Their study suggested that protein replacement restored learning and memory function to similar levels compared to wild-type mice. Furthermore, therapy also drastically reduced episodes of apnoea to levels similar to control mice and also significantly decreased clasping. They also established that CDKL5 replacement restores the hippocampal structural defects that characterize Cdkl5 KO mice, and that this effect extends beyond the cessation of treatment.
Finally, the authors wanted to establish whether these beneficial effects could be seen if protein replacement was injected systemically. The TAT-CDKL5 complex was therefore injected under the skin of baby mice and intravenously into adult mice. In both instances, they were able to identify the presence of replacement protein in the brains of the mice, suggesting that the protein complex can cross the blood-brain-barrier. They subsequently showed that this led to restoration of neuroanatomical and behavioral defects in Cdkl5 KO mice, and that both visual function amelioration and improvement of histological features - spine density and PSD-95 puncta staining - outlasted the period of treatment. This suggests that discontinuous protocols of treatment could be effective. More studies will be needed to determine whether this approach can be effective in individuals with CDKL5.

# Supporting CDKL5

**The antidepressant tianeptine reverts synaptic AMPA receptor defects caused by deficiency of CDKL5.**
**Marco Tramarin et al.**
**Human molecular genetics 27.12 (2018): 2052-2063.**

An AMPA receptor is a receptor found in the Central Nervous System. It is present in the cell wall and is thought to medicate rapid transmissions across the synaptic junctions between neurons. The receptor consists of 4 sub-units. In this study, the authors identify that in the Cdkl5 KO mouse, the expression of one of these sub-units is down regulated and that expression of the AMPA receptor at the surface is then also impaired. The hypothesis is that this impairment may, in part, lead to altered synaptic function which may in turn contribute to the cognitive impairment seed in CDKL5. The authors go on to shown that these defects in the AMPA receptor can be reversed with Tianeptine. Tianeptine is an antidepressant originally developed in France and is currently only licensed for use in certain countries. This study suggests a potential role in the treatment of CDKL5.

**Treatment with the GSK3-beta inhibitor Tideglusib improves hippocampal development and memory performance in juvenile, but not adult, Cdkl5 knockout mice.**
**Claudia Fuchs et al.**
**European Journal of Neuroscience, pp. 1–13, 2018.**

It has been established that in the Cdkl5 KO mouse that following birth, Cdkl5 deficiency causes defects in the development of part of the brain called the hippocampus and consequently the learning and development associated with this part of the brain. The authors also note that these structural defects were associated with increased activity of GSK3b, which is an important inhibitory regulator of many neuronal functions. Tideglusib is a GSK inhibitor and is under investigation for a number of neural and neuromuscular disorders. In this study, the authors demonstrate that its use improves the histological and clinical features associated with the hippocampal defects seen in the Cdkl5 KO mouse. This improvement was only observed in juvenile mice. The authors also point out that GSK3b has a wide range of substrates and therefore there may be a number of pathways involved in the improvement seen in Cdkl5. However, they suggest that treatment strategies aimed at normalising GSK3b activity might be of value in CDKL5.

# Supporting CDKL5

**Comprehensive behavioural analysis of the *Cdkl5* knockout mice revealed significant enhancement in anxiety- and fear-related behaviors and impairment in both acquisition and long-term retention of spatial reference memory.**
**Kosuke Okuda et al.**
**PloS one 13.4 (2018): e0196587.**

The title more or less sums up the results of this study in which various behaviors of Cdkl5 KO mice are compared to those of wild type. When compared to a control group of normal "wild-type" mice, Cdkl5 KO mice show

- normal muscular strength and sensory activities
- a mild alteration in gait
- significantly enhanced anxiety-type behaviours
- a uniquely altered social interaction, in that when Cdkl5 KO mice are exposed to an unfamiliar mouse in a novel environment, the mice avoided exploring and preferred staying with each other
- significant impairment of both acquisition and long-term retention of spatial reference memory
- impaired working memory
- Impaired dendritic arborization and immature spine development of hippocampal pyramidal neurons

So, a fairly detailed behavioural analysis of Cdkl5 KO mice. The authors also affirm that "All experiments were performed in accordance with the national guidelines and approved by the Animal Experiment Committees of Graduate School of Medicine, the University of Tokyo".

## Living with CDKL5

**A framework for understanding quality of life domains in individuals with the CDKL5 deficiency disorder.**
**Jodilee Tangarorang et al.**
**American Journal of Medical Genetics Part A 179.2 (2019): 249-256.**

This study from the International CDKL5 Disorder Database was aimed at identifying the quality of life (QOL) domains important for individuals with CDKL5. Twenty-five parents of individuals registered in the database participated in semi-structured telephone interviews to explore areas that supported or challenged their child's QOL.
Overall, physical health was primarily affected by seizures and this was said to be extensively discussed by the parents. Gastro-intestinal problems and sleep disturbance, which are common in CDKL5, are said to further compound poor health status. Because children with CDKL5 are generally non-verbal, they rely on caregivers to recognise their body language, facial expressions and vocalisations to make their needs known.
This is the first study to investigate QOL in CDKL5 and it provides insight of the complexities of daily life living with an individual who has CDKL5. It is hoped that the framework provided will help to guide counselling for families soon after diagnosis, and will also assist health professionals, and other service providers planning ongoing support and management for affected families.

# Supporting CDKL5

**Open-label use of Highly* purified CBD (Epidiolex®) in patients with CDKL5 deficiency disorder and Aicardi, Dup15q, and Doose syndromes.**
**Orrin Devinsky et al.**
**Epilepsy & Behavior 86 (2018): 131-137.**

This open-label drug multi-centre trial provides some provisional evidence for the long-term safety and efficacy of cannabidiol (CBD) administration in patients with treatment-resistant epilepsy (TRE) associated with CDKL5. The study also looks at individuals with Aicardi, Dup15q, and Doose syndromes. There were 18 females with CDKL5 with complete data, 16 of whom were aged under 18 years with 2 above. CBD was added as an addition to their normal anti-epileptic medication (AED). There were 10 on 1 or 2 AED's while 4 were on 3 and another 4 on 4 or more AED's. Baseline seizure activity was recorded over a 4 week period following which CBD was administered, initially at 5 mg/kg/day in two divided dosages . This was increased gradually until intolerance or a maximum dosage of 25 mg/kg/day was achieved – although some treating centres allowed higher doses to be achieved. Resultant seizure activity was recorded at 12 weeks and again at 48 weeks. Responders at each time period were defined as subjects whose mean reduction in monthly convulsive seizure frequency was 50% or greater.
Overall, in the CDKL5 group, 41% reported a greater than 50% reduction in seizure activity at 12 weeks Thish had improved to 53% by 48 weeks. In their safety analysis, the most frequently reported adverse events were diarrhea, somnolence, and fatigue. Serious treatment-emergent adverse events included convulsion (9%), status epilepticus (9%), and respiratory infection (5%). The authors suggest that further controlled trials should be undertaken.

**A male case with CDKL5-associated encephalopathy manifesting transient methylmalonic acidemia.**
**Satoshi Akaminea et al.**
**European journal of medical genetics 61.8 (2018): 451-454.**

Methylmalonic acidemia (MMA) is a genetic disorder in which the body cannot break down certain proteins and fats. The result is a build up of a substance called methylmalonic acid in the blood. Clinical features usually appear in early infancy and can be mild or life-threatening. Features include vomiting, dehydration, weak muscle tone (hypotonia), developmental delay, lethargy, an enlarged liver and failure to thrive. The author present a case report of a 16 year old boy with a truncating CDKL5 mutation in exon 7 who developed a transient episode of MMA. The normal genetic causes associated with this condition were not present and the authors therefore suggest that this transient abnormality occurred as a result of deranged CDKL5 protein function. Further studies are suggested.