CDKL5 Research 2019

The year 2019 saw the publication of over 30 studies on CDKL5. These ranged from the study of the basic molecular biology of CDKL5, the search for potential treatments and the development of clinical methods of assessing the severity of CDKL5 and the outcome of treatment. Research into CDKL5 has really started to accelerate and much of this is due to the support of the Loulou Foundation which has now funded about 40 research projects into CDKL5. Here, I have summarised some of the studies that I have been able to access. It is not a comprehensive review and I have tended to "gloss over" some of the science which is becoming more complex as the fundamentals of CDKL5 become known. This is really aimed at busy parents and carers who may not have had chance to catch up with research and would like an idea of what is currently happening. Hope it helps...

Epidemiology

The incidence of CDKL5 Deficiency, that is, the frequency of newly diagnosed cases, has not previously been really known. The first insight into this has come from a multi-centre study from Scotland **(1)** where the incidence was determined for a number of childhood-onset genetic epilepsies including CDKL5. For CDKL5, the incidence was determined to be 1 in every 42400 live births which is the equivalent of 2.36 cases per 100 000 births. Given that there may have been some individuals who were missed by the study, the authors suggest that this reported incidence should be considered a minimum estimate.

The CDKL5 Protein

Mitochondria are the power stations of cells. By converting oxygen into ATP, they produce the chemical energy that drives cellular functions. Abnormalities of their function has previously been described in relation to Rett Syndrome and similar abnormalities are described in CDKL5. In this study **(2)**. Skin cells were taken from a patient with CDKL5 and converted into neural progenitor cells (cells destined to develop into nerve cells).The authors show that in these cells mitochondrial function is abnormal as indicated by relatively high oxygen consumption rates. They also displayed altered electrophysiology and the mitochondria themselves looked abnormal. These features are therefore thought to be common to CDKL5 and Rett syndrome.

Neurons (nerve cells) in the brain can be classified in different ways. One way is to define whether they excite (stimulate) or inhibit other neurons around them. Excitatory neurons often use a neurotransmitter called glutamate and are therefore known as glutatmatergic neurons while inhibitory neurons produce an amino acid called GABA and are therefore called GABAergic neurons. The expression of *cdkl5* is particularly prominent in these 2 types of neurons in the forebrains of mice. In a knockout (KO) mouse the whole cdkl5 gene is removed from all neurons. However, it is also possible to selectively ablate *cdkl5* expression in specific cell types. Previous studies have shown that mice lacking *cdkl5* in glutamatergic neurons display deficits in learning and memory similar to that seen in CDKL5 but not other behavioural deficits such as alterations in sterotypic and anxiety-related behaviour or in sociability. This study **(3)** has now shown that where *cdkl5* is selectively removed from GABAergic neurons in the forebrains of mice then these mice exhibit autistic-like behaviour but interestingly learning and memory functions appear preserved. Other signalling mechanisms are involved but the message appears to be that the different clinical characteristics that we see in CDKL5 may be individually related to the loss of the *cdkl5* protein in specific neuron populations. A

further similar study **(4)** has also shown that loss of *cdkl5* alters what is known as mTOR signaling as well as synaptic compositions in a neuron-type specific manner. These results support the developing view that the *cdkl5* protein may have distinct functional roles related to cellular signaling in excitatory and inhibitory neurons and that CDKL5 Deficiency may have distinct neuron-type specific origins and effects.

Cortical Visual Impairment (CVI) is a recognised characteristic of CDKL5. In this study **(5)** the authors investigate the basis of this in the mouse model. The visual pathways begin in the retina of the eye from where visual neurons pass backwards through the brain. They then pass through something called the Dorsal Lateral Geniculate Nucleus (DLGN) which is basically the main relay station on the way to the primary visual cortex (PVC) at the back of the brain – this is where the brain synthesises the electrical impulses from the eye into the images we perceive. The authors first studied the visual pathways in a KO mouse. They found no obvious abnormalities in the retina, but did identify morphological abnormalities in dendritic spines in the DLGN and PVC. Secondly, they selectively deleted *cdkl5* from excitatory cells in the PVC. This produced abnormalities in visual cortical responses which suggests that normal function of visual cortical circuits is *cdkl5* dependant.

Potential Drug Therapies

There is increasing research being undertaken into drug therapies for CDKL5. Some research is at the early stage of identifying potential therapeutic agents whilst other research is at the clinical trial stage.

In the production of the *cdkl5* protein, the gene is initially copied (transcribed) to produce what is called the primary transcript (RNA). This primary transcript is then spliced to produce the mRNA which is subsequently used to generate the final protein though translation. Therapies aimed at curing CDKL5 have centred around gene replacement or editing and protein replacement therapy. However, there is evidence that cellular levels of the *cdkl5* protein are finely tuned and regulated, and the authors here **(6)** suggest that this could prove an obstacle to the effectiveness of these therapies. Some CDKL5 mutations affect the splicing stage of protein production and in this rather technical paper the authors explore the potential of an alternative therapy which involves correcting the pre-mRNA splicing through the use of another small RNA molecule, called U1snRNA. They suggest that about 15% of gene variants associated with CDKL5 would be amenable to this form of therapy. They also state that if successfully developed, such therapy would preserve the normal gene regulation processes thus avoiding the potential problems with fine tuning of the *cdkl5* protein.

This review **(7)** looks at the evidence for the use of cannabis in the treatment of CDKL5-related epilepsy. It is only relatively recently that its role has been investigated, with the 2 major compounds best characterised being tetrahydrocannabinol (THC) and cannabidiol (CBD). Studies have described the anticonvulsant effects of cannabinoids including CBD in a variety of preclinical animal models while the anticonvulsant effects of THC have also been demonstrated in mice. Furthermore, cannabinoids may be beneficial for more than just seizures, with CBD also reducing autistic-like social deficits in a mouse model of Dravet syndrome. Specific evidence for its use in CDKL5 is anecdotal with inadequate long-term follow-up. Most of the evidence for using cannabis in CDKL5 is based on research in similar early-onset epileptic encephalopathies such as Dravet syndrome and Lennox–Gastaut syndrome (LGS). Therefore, before cannabis can be used to treat CDKL5-related epilepsy much more data is needed on both the short and long term efficacy and safety.

In nonsense mutations, a stop codon is produced prematurely. There are 3 potential stop codons that can be produced and the subsequent *cdkl5* protein is truncated. Aminoglycosides are a class of antibiotic which have also been found to overcome premature stop codons. A study from the University of Milan **(8)** has found that the aminoglycosde gentamicin is effective at reading through all 3 stop codons that can occur and therefore can produce a full length protein. However, while the localisation of the *cdkl5* protein around the cell appears relatively normal, its kinase activity is considerably diminished raising some questions regarding how useful this approach will be for the future.

A number of other studies have looked at the molecular biology of the *cdkl5* protein in an attempt to identify potential future therapeutic agents. Here are a few...

In one study **(9)** a group reported on a knock-in mouse study. A knock-in (KI) mouse is where a specific mutation is inserted into the mouse's DNA. They produced a mouse with the nonsense mutation R59X and showed that it displayed similar behavioural changes to those seen in knockout (KO) mice. Furthermore they were able to show that there were associated abnormalities in the regulation of a specific AMPA receptor to which the neurotransmitter glutamate binds and which is thought to play a role in the development of memory. By correcting this abnormality pharmacologically with a molecule called IEM-1460 the authors saw improvements in the behavioural changes of the mice.

The perihinal cortex (PRC) is thought to be an important region of the brain for memory. In this KO mice study **(10)** processes thought to be involved in laying down memory were abnormal. Neurons in the PRC appeared abnormal and there were also abnormalities of the same AMPA receptors mentioned in the study above. Again most of these abnormalities could be reversed pharmacologically with a molecule called R13 suggesting another therapeutic strategy for further investigation.

Substrates are those molecules on which enzymes act and much research is being done to identify the substrates of *cdkl5.* In this study **(11)** the authors identify one such molecule called SMAD3. CDKL5 mutations cause abnormalities in the activity of SMAD3 and this leads to the death of developing neurons. This abnormal activity can be overcome with the use of another molecule called TGF-β1 which in turn rescues the survival of neurons.

A number of previous studies have suggested the possibility that CDKL5 could be classed as a ciliopathy. A cilia is a tubular structure associated with many functions in the human body. If you think about it, neurons are tubular structures whose development may involve processes similar to that of cilia and in which the *cdkl5* protein may play an important role. A review from Italy **(12)** looks at the link between *cdkl5* and what are called microtubule-binding proteins and suggests how *cdkl5* deficiency could lead to deranged neuronal function. The authors propose that the understanding of this may lead to the development of therapeutic approaches in the future.

Most mouse studies on CDKL5 have been on young mice. In this study **(13)** the researchers looked at the behaviour of older mice, aged 9 to 12 months, and identified a number of behavioural changes. They also studied something called Prepulse Inhibition (PPI) which is a neurological phenomenon seen in many species including humans and mice. Abnormalities of PPI have been noted in patients with schizophrenia and Alzheimer's. The researchers here observed severe PPI deficits in KO mice. These behavioural changes and the deficits in PPI were reversed with the use of a molecule called LP-211 which is a selective agonist (activator) of the serotonin receptor 7 (5-HT7R). This receptor is broadly expressed in the central nervous system and is said to be involved in a variety of

neurophysiological phenomena relevant to CDKL5, such as sleep, cognitive processes and synaptic plasticity. They also noted abnormal mitochondrial function in the KO mice, an abnormality which was again restored to normal by treatment with LP-211.

Living with CDKL5

Quality of life (QOL) measures are becoming increasingly important in the assessment of long-term conditions and in particular, the impact of treatment on those conditions. They are made up of different domains which may vary between conditions. They typically represent a measure of an individuals perception and satisfaction of aspects of their own life. This paper **(14)** describes a study that is the first to identify QOL domains for individuals with CDKL5. The study is drawn from interviews with 25 families identified through the International CDKL5 Database, established in 2012. There were 20 females and 5 males with an age range of 3 to 35 years. Of the 25, 17 were unable to walk, only 4 could communicate through words and only 2 were seizure free. All those studied were considered to have pathogenic mutations, although the types of mutation varied. Data was acquired through semi-structured telephone interviews with families, and considerable emphasis was placed on minimising misrepresentation to ensure accuracy of the data. Analysis of the data was based on previous QOL domains used in Rett syndrome.
The 10 QOL domains identified for CDKL5 fall into 3 categories;

Category	Domain
Health and Well being	physical health
	bodily pain and discomfort
	behavioural and emotional well being
Daily activities	communication
	movement and mobility
	stability of routines
Community immersion and services	social connectedness
	nature and outdoors
	variety of activities
	provision and access to services

Examples are given for each domain through parents quotes. This is the first study to examine those QOL domains that may be important to individuals with CDKL5. The hope is that this will provide a valuable reference for health professionals, carers and other support workers to assist in the development and well being of individuals with CDKL5 from a holistic point of view.

Severity Assessment (SA) tools have been developed in clinical practice for use in decision making and indicating prognosis. Up to now, no such SA tool has been available for CDKL5. In this study **(15)** the authors present a clinical tool for assessing the severity of individuals with CDKL5. This involved a multi-centre approach and the review of clinical and research data of 111 individuals with CDKL5. Contributions were supplied through the available literature, the clinical and research experience of an international panel of experts and the live experience of parent participants. Once an initial assessment score had been developed, the SA went through a number of review cycles before the current SA form was achieved.

The assessment consists of 8 Domains, 1-Epilepsy, 2-Motor, 3-Cognition and Vision, 4-Autonomic, 5-Overall Impression, 6-Therapies with 2 further Domains for scoring and clinical decision making which are completed by the attending clinician. It is hoped that this new Severity Assessment tool will be taken up and used by clinicians treating individuals with CDKL5.

A study from the US **(16)** looked at determining whether there was a relationship between the cdkl5 mutation, the seizure type, the presence of cortical visual impairment (CVI) and development. They studied 92 individuals with CDKL5 of whom 18 were male. They found no correlation between mutation and the clinical variables they were studying. Cortical visual impairment was identified in 70 (76%) patients. Although there was no correlation with genotype there was an association between CVI and poor milestone development.

In a separate study **(17)** researchers studied pain perception both clinically and the potential underlying causes. They analysed data collected in the CDKL5 International Registry and Database. They identified that altered pain sensitivity in children with CDKL5 was reported by 53% (122/230) of their carers. Of those 122 children reported as displaying alterations in pain sensitivity 70 (57.4%) were reported as displaying reduced pain sensitivity, 24 (19.7%) enhanced sensitivity and 28 (22.9%) both reduced and enhanced sensitivity. Reduced pain sensitivity was more likely in those 6 years of age or over compared to those aged 2 years or younger, and in those whose mutations were in the kinase (catalytic) domain of the CDKL5 gene. Up to now, all *cdkl5* molecular studies have focused on neurons in the brain (central nervous system). However, through mouse and cell culture studies, the authors went on identify that the *cdkl5* protein is also present in the cell bodies of peripheral sensory nerves (called the Dorsal Root Ganglion) where it appears to have a role in pain pathways. This might explain reduced pain sensitivity. Enhanced pain sensitivity, on the other hand, might be related to increase excitability of the central nervous system and abnormal processing of pain signals.

This study **(18)** reported on 8 patients (7 females and 1 male) out of 14 with CDKL5 on whom sufficient clinical and therapeutic data was available. In particular, the authors focused on parameters around seizure activity and related video and EEG characteristics. Two of the patients had both synchronous EEG and video recordings of their first seizure type with one also having deltoid EMG studies. The authors propose that this data suggests a new previously unreported seizure pattern consisting of a tonic phase followed by a clonic phase and then spasms. They also noted that seizures in these 2 patients and 3 more responded to the combination of vigabatrin and zonisamide.

A study from Korea **(19)** looked at the association between the mutation and the effectiveness of anti-epileptic treatment. - namely anti-epileptic drugs, the ketogenic diet and steroids. They presented data on 8 females and 2 males with CDKL5. All but one had abnormal EEG's of whom 2 displayed hypsarrthmias. Only 1 (female) showed improvement (>50% reduction) in seizure activity with anti-epileptic medication, 5 of the non-responders were on triple therapy. Out of 8 patients who were on the ketogenic diet only 1 showed any benefit (<50% reduction) in seizure activity. Of the 7 who were treated with steroids, 3 showed >50% reduction in seizure activity whilst 1 remained seizure free for more than 6 months. This was a relatively small retrospective study and the period of follow-up is unclear.

In a "Letter to the Editor" in a Canadian journal **(20)** the author describes reflex seizures in an infant with CDKL5. The seizures occurred in response to water immersion when being bathed and were first noticed at 6 months of age. Her seizures subsequently progressed to occur unprovoked.

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