Supporting CDKL5

CDKL5 Research 2020

Research into CDKL5 has continued to provide more information about the underlying molecular pathways in which CDKL5 is involved as well as the spectrum of the clinical presentation. More research is being done into therapeutic options, both in terms of managing symptoms, especially seizures, as well as looking at potential strategies to correct the underlying disorder, particularly through gene engineering.

While CDKL5 has been recognised as a distinct clinical entity for some time, this has now been formalised. CDKL5 has become known as CDKL5 Deficiency Disorder (CDD) and on the 1st October 2020 it was given its own ICD-10 code (International Statistical Classification of Diseases and Related Health Problems). CDD is now designated G40.42 by the World Health Organisation (WHO).

Considerable funding has been provided by the LouLou Foundation and we are seeing more focused research. This is not a detailed analysis of this year's research on CDD. It is more of a short summary of some of the key messages that have come out of published papers. As usual, I have listed the papers for further reading and included those that have not been summarised here but may be of interest.

Mouse Studies and Biology

In a previous study from Taiwan, the authors demonstrated behavioural changes in Cdkl5 KO mice (Cdkl5 KO mice is where the Cdkl5 gene has been removed from the X chromosome). These behavioural changes were similar to autism-like features (impaired communication and sociability) and ADHD features such as hyperlocomotion. In this study (1), the same group suggest an underlying molecular disorder for these observed behavioural changes. They studied a part of the brain called the striatum, which has a role in movement, and identified that CDKL5 is required in certain types of neurons, called dopaminergic neurons – see Supporting CDKL5 (supporting-cdkl5.co.uk) - to supress hyperlocomotion. This may occur through regulation of the gene expression of dopaminergic proteins by CDKL5.

In a paper from Italy (2) the authors present CDKL5 studies on cells derived from a human (neuroblastoma) cell line. They first show that CDKL5 deficiency in this cell line produces abnormalities in neurite outgrowth during neuronal differentiation previously seen in Cdkl5 KO mice. They then show that CDKL5 deficiency in these human cell lines is associated with a decrease in cell proliferation and an increase in cell death through the deregulation of certain signalling pathways. The authors suggest that CDKL5 deficiency may ultimately impair DNA repair mechanisms leading to DNA damage. Further research needed.

We know from previous studies that the appearance of neurons in Cdkl5 KO mice are abnormal when compared to those in normal (wild-type) mice. The mechanism

by which neurons develop involves the formation of microtubules and this process is influenced by CDKL5 through the involvement of something called CLIP170 which regulates the development of microtubules. Previous studies have also identified that the neurosteroid pregnenolone can reverse these observed defects. In this study (3) the authors identify that pregnenolone and a synthetic derivative pregnenolone-methyl-ether (PME) can restore neuronal structure through CLIP170, raising the possibility of a therapeutic use. PME in particular, may be useful as it is not metabolised to a further active substance and so may provide a safer therapeutic approach.

Part of the clinical features of CDD relate to impaired sensory perception and processing. These features are also seen in mouse models of CDD. In this paper (4) the authors look at potential underlying causes by studying those specific pathways that transfer incoming sensory stimuli to the cortex. The whiskers of mice are incredibly sensitive and are said to be the equivalent of human fingertips. Here, the authors studied and compared the associated sensory neural pathways in the brains of Cdkl5 KO and wild type (WT) mice. They found derangement of synapses within these sensory neural pathways in KO compared to WT mice. This was associated with atypical behavioural response to whisker-mediated tactile stimulation. Interestingly, putting the KO mice into what is basically described as an enriched sensory stimulating environment not only restored normal behavioural responses in them but also re-established synaptic organisation within their sensory neural pathways. The implication of this work is that although children with CDD have significant difficulty in processing sensory information, a programme of enhanced sensory stimulation may potentially go some way to overcoming this – a strategy that perhaps any family with the right advice could begin immediately.

The central nervous system consists of the brain and spinal cord and previous research into CDKL5 has focussed on the brain. The nervous system outside of that is called the peripheral nervous system, and in this study (5) the authors identify that CDKL5 has a role in the regulation of pain perception (nociception) in the peripheral nervous system. Dorsal root ganglia (DRG) contain the cell bodies of peripheral sensory neurons and are usually located just outside the spinal cord. The authors have identified that CDKL5 is selectively expressed both in the DRG of mice and in human iPS-derived nociceptors - see Future Research Supporting CDKL5 (supporting-cdkl5.co.uk) - and is required for the growth of sensory neurons and regulation of nociceptive signalling pathways. Blocking its action interferes with these processes and the authors observed reduced behavioural responses in mice to nociceptive stimuli. The authors were able to show a clinical corollary with their study by acquiring data from the International CDKL5 Database. This showed that 53% (122/230) of caregivers reported altered pain sensitivity in their children. Of these 57.4% (70/122) specifically reported decreased pain sensitivity, 19.7% (24/122) reported enhanced pain sensitivity whilst 22.9% (28/122) reported both. The authors suggest that monitoring pain perception may be a way of testing disease-modifying treatments in a pre-clinical setting and that future gene therapies should also be aimed at the peripheral and not just central nervous system.

If you are a green tea drinker, then you might be aware that it contains compounds called catechins which are said to have numerous health benefits. The main catechin in green tea is called epigallocatechin-3-gallate (EGCG). What has this to do with CDD I hear you ask? Well, in this study (6) the authors have identified that EGCG can restore the synaptic and dendritic developmental defects seen in Cdkl5 KO mice. Furthermore, such defects in the brains of adult KO mice can also be restored through the intraperitoneal injection (into the abdominal cavity) of EGCG although the associated behavioural deficits did not improve. The authors further identified that this mechanism of action was likely to occur through the downregulation of another compound called DYRK1A which they found to be upregulated in Cdkl5-KO neurons. These results indicate the potential for further therapeutic investigation. More green tea vicar?

One of the conspicuous features of mouse models of CDD has been the lack of seizure activity. In this study (7) the authors have been able to identify seizure activity in relation to mosaicism. To recap on mosaicism – female cells have 2 X chromosomes (XX) which is termed homozygous (2 normal X's). If one X carries a mutation – Xm – then the female is said the be heterozygous (XXm – 1 affected X). Due to X-inactivation some cells will use the normal X chromosome while others will use the mutated Xm chromosome. You then basically have 2 subsets of cells that either use the normal X or the mutated Xm – that is mosaicism. Because males only have 1 X chromosome (XY) if they also have a mutation (XmY) then because X-inactivation does not occur in males, all their cells are affected and they are said to be hemizygous. In this study the authors observed that only heterozygous female mice developed seizure activity whereas homozygous females and hemizygous males did not. Seizure activity developed in both heterozygous knockout mice (KO/X) and in heterozygous knockin mice (R59X/X) where a specific mutation (R59X) was inserted into the mice gene (keep up!!). Seizures did not occur in homozygous knockout female mice (KO/KO) or in hemizygous males (KO/Y or R59X/Y). So, seizures only occurred in heterozygous females and the onset of seizure activity was generally much later (when the mice were effectively in middle age) compared to the early onset seizures seen in humans. There was also some evidence that heterozygous females (R59X/X) had a limited lifespan compared to their wild-type (X/X) littermates. No such reduction in lifespan was seen between hemizygous males (R59X/Y) and their littermates (X/Y). The authors hope that further research here will help us understand the mechanisms underlying CDD-related epilepsy.

Non-neuronal effects of CDKL5

In one of the first papers to look at the effects of CDKL5 deficit outside the nervous system, this paper suggests a role for CDKL5 in the regulation of renal cells. The function of our kidneys is considerable and varied and reflected in their complex anatomical structure. Cells called renal tubular epithelial cells (RTEC) are implicit to this process. Kidney damage also called Acute Kidney Injury (AKI) can occur due to a number of conditions. In this study (8), the authors suggest that while normal renal

function is not dependent on the presence of CDKL5, in the presence of AKI CDKL5 does have a role in the process of renal cell dysfunction and death (reflecting some of the observations in paper 2). A second related study (9) identifies these effects of CDKL5 on AKI through its suppression of a "protective" regulator called SOX1. This raises the question of whether "anti-CDKL5" therapy could be used to treat AKI and what effect on the CNS this might have?

Gliomas are the commonest type of malignant brain cancer and are derived from glial cells which are supporting cells found between neurons. This study (10) from China has found that CDKL5 has a role in the way gliomas grow and spread. CDKL5 is highly expressed in glioma cells and is involved in their proliferation and invasion. CDKL5 also appears to promote resistance against a molecule called β-lapachone which is known to have anti-cancer effects.

Long non-coding RNA's are types of transcript that are not thought to be translated into proteins. They are thought to have a number of regulatory roles. One in particular, Long Intergenic non-protein coding RNA 680, or LINC00680, has been found to be a prognostic indicator in soft tissue tumours and also to promote the progression of non-small cell lung cancer and glioblastoma cells. In this paper (11) the authors study factors involved in drug-resistance breast cancer. In particular, they looked at resistance to a drug called Docetaxel used to treat several cancers including breast cancer. They found that LINC00680 promotes cell proliferation, migration, and invasion in breast cancer. Furthermore, it may achieve this and promote resistance to treatment with docetaxel through a CDKL5 pathway. More to come, I suspect!

Gene Therapy

X-linked conditions, such as CDD, are affected by X-inactivation. Therefore, one line of therapy that has been considered is the re-activation of the normal CDKL5 gene in cells where the mutated gene is being expressed. In this study (12), the authors have investigated a potential mechanism through which this could be achieved. When a gene is being read (transcription) the start of the gene is typically marked by a promoter region. DNA methylation is a process through which the expression of a gene is suppressed. For the inactive CDKL5 gene, inactivation involves DNA methylation of its promoter. By removing methylation of the promoter using a "fusion protein" the authors demonstrated an increase in the expression of the previously inactivated CDKL5 gene. Using methods derived from CRISPR technology (a technique for repairing gene mutations), they were able to specifically target the CDKL5 gene for re-activation. Early days but great potential for future therapies.

Clinical Studies

In a clinical review (13) of 3 female children with CDD, the authors describe electroclinical and neurodevelopmental features that have been identified in previous studies. In addition to the early onset of seizures, they observed that a long "honeymoon" period of seizure free activity correlates with better epilepsy outcome. Furthermore, as with other studies, they observed no association between the course of the epilepsy and neurodevelopmental delay, thus restating a view that epilepsy should be considered as part of the condition rather than as a cause of the neurodevelopmental delay. In a further case report (14), the authors describe a female with typical features of CDD. Her mutation has been found in one other person and the authors suggest more research into genotype-phenotype correlation is required.

A publication from China (15) reported 3 patients with CDD who each had a previously undescribed mutation that were thought to be pathogenic. One was a truncating mutation in exon 15, one a substitution in exon 6 and the last a frameshift mutation also in exon 6 - see Supporting CDKL5 (supporting-cdkl5.co.uk).

In this case report (16) from the International CDKL5 Disorder Database, a 29-year-old female is described who has a mutation in the kinase domain of the CDKL5 gene but does not have all the hallmarks of CDD. Although she displayed some of the typical facial characteristics and also experienced teeth grinding, she had never suffered epileptic seizures and had more advance communication and motor skills than might be expected. In particular, she could talk in sentences, read, write, walk, run and ride a bike. She did experience periods of altered mental state, confusion, aggression, repetitive behaviour and palilalia 3 to 4 times a year, each lasting one day to 3 months. Afterwards she has no recall of these episodes. Despite her relatively high function, computer analysis identified her mutation as being pathogenic. However, although the expressed CDKL5 protein had markedly reduced kinase activity compared to "normal" levels, it was still above that seen in more severe forms of CDD.

In another study (17) the authors assessed the possibility of a genotype-phenotype relationship using 2 outcome measures. The CDKL5 Development Score (CDS) has been developed to assess the level of development of an individual with CDD while the CDKL5 Clinical Severity Assessment (CCSA) is used to assess how severely an individual is affected by CDD. The authors found that specific mutations were associated with very different degrees of development and severity, even mutations within a short-defined area of the gene produce varying phenotypes. So, it would appear that specific pathogenic mutations may be associated with differing degrees of reduction in CDKL5 kinase activity. These may be further affected by other factors, such as the degree of X-inactivation, which all goes to influence the severity of the clinical presentation of CDD.

A further study from the International CDKL5 Disorder Database (18) is the first to explore the Quality of Life (QoL) in individuals with CDD. QoL was assessed using

the QI-Disability measure which has 6 domains (physical health, positive emotions, negative emotions, social interaction, leisure and independence). The authors found that functional impairments such as the inabilities to sit, communicate or use hands had the greatest impact on QoL. The authors noted some correlation between QoL and genotype with those individuals who had truncating mutations between amino acids 172 and 781 (found in exons 8 to 16) having a better QoL in their physical health domain. Individuals with more frequent seizure activity also generally had poorer QoL. However, the effect of anti-epileptic medication outweighed the effect of seizure frequency particularly in the physical health domain so that individuals who were on one epileptic medication had better QoL than those on three or more. The authors also noted some geographical variations with those living in North America generally having a better QoL than those living in Europe, Australia, New Zealand and a few other “miscellaneous” countries. Some socio-economic reasons for this are suggested.

A form of seizure activity called infantile spasms (IS) can occur early in CDD and may be the presenting feature. In this poster presentation (19) the authors report on the response of IS to first line treatments in patients with CDD compared to those with other conditions. They found that IS in patients with CDD generally responded poorly. The number of patients in this study were relatively small and clearly more data is needed but it does go some way to emphasising the difficulties faced when treating seizure activity in CDD.

In a poster presentation (20), the authors report the early results of the Marigold study on Ganaxolone. This is a randomised double-blind trial and has recruited 101 patients in 8 countries. The take home message so far is that a greater than 50% reduction in seizure activity has been seen in 32.2% of patients on Ganaxolone compare to 4% on placebo. Ganaxolone was generally well tolerated with a less than 5% discontinuation rate – the commonest adverse event being somnolence.

Martyn Newey
CDKL5 Dad
December 2020.
www.supporting-cdkl5.co.uk

References

1. Jhang, Cian-Ling, et al. "Dopaminergic loss of cyclin-dependent kinase-like 5 recapitulates methylphenidate-remediable hyperlocomotion in mouse model of CDKL5 deficiency disorder." *Human Molecular Genetics* 29.14 (2020): 2408-2419.
2. Loi, Manuela, et al. "Increased DNA Damage and Apoptosis in CDKL5-Deficient Neurons." *Molecular neurobiology* (2020): 1-19.
3. Barbiero, I., et al. "Pregnenolone and pregnenolone-methyl-ether rescue neuronal defects caused by dysfunctional CLIP170 in a neuronal model of CDKL5 Deficiency Disorder." *Neuropharmacology* 164 (2020): 107897.
4. Pizzo, R., et al. "Structural Bases of Atypical Whisker Responses in a Mouse Model of CDKL5 Deficiency Disorder." *Neuroscience* 445 (2020): 130-143.
5. La Montanara, Paolo, et al. "Cyclin-dependent–like kinase 5 is required for pain signaling in human sensory neurons and mouse models." *Science Translational Medicine* 12.551 (2020).
6. Trovò, L., et al. "The green tea polyphenol epigallocatechin-3-gallate (EGCG) restores CDKL5-dependent synaptic defects in vitro and in vivo." *Neurobiology of Disease* 138 (2020): 104791.
7. Terzic, Barbara, et al. "X-linked cellular mosaicism underlies age-dependent occurrence of seizure-like events in mouse models of CDKL5 deficiency disorder." *Neurobiology of Disease* 148 (2020): 105176.
8. Kim, Ji Young, et al. "A kinome-wide screen identifies a CDKL5-SOX9 regulatory axis in epithelial cell death and kidney injury." *Nature Communications* 11.1 (2020): 1-15.
9. Kim, Ji Young, et al. "Involvement of the CDKL5-SOX9 signaling axis in rhabdomyolysis-associated acute kidney injury." *American Journal of Physiology-Renal Physiology* 319.5 (2020): F920-F929.
10. Jiang, Zhenfu, Tongtong Gong, and Hong Wei. "CDKL5 promotes proliferation, migration, and chemotherapeutic drug resistance of glioma cells via activation of the PI3K/AKT signaling pathway." *FEBS Open Bio* 10.2 (2020): 268-277.
11. LI, JIA, et al. "LINC00680 Modulates Docetaxel Resistance in Breast Cancer via miR-320b/CDKL5 Axis." (2020).
12. Halmai, Julian ANM, et al. "Artificial escape from XCI by DNA methylation editing of the CDKL5 gene." *Nucleic acids research* 48.5 (2020): 2372-2387.
13. Bernardo, Pia, et al. "Clinical evolution and epilepsy outcome in three patients with CDKL5-related developmental encephalopathy." *Epileptic Disorders* 21.3 (2019): 271-277.
14. Takeda, Kanako, et al. "Clinical features of early myoclonic encephalopathy caused by a CDKL5 mutation." *Brain and Development* 42.1 (2020): 73-76.
15. Yan, Yumei, et al. "Novel CDKL5 mutations were found in patients in China: retrospective investigation in cases of CDKL5-related disorders." *Italian Journal of Pediatrics* 46.1 (2020): 1-6.
16. MacKay, Conor I., et al. "Expanding the phenotype of the CDKL5 deficiency disorder: Are seizures mandatory?." *American Journal of Medical Genetics Part A* 182.5 (2020): 1217-1222.
17. MacKay, Conor I., et al. "Exploring genotype-phenotype relationships in the CDKL5 deficiency disorder using an international dataset." *Clinical Genetics* (2020).
18. Leonard, Helen, et al. "Exploring quality of life in individuals with a severe developmental and epileptic encephalopathy, CDKL5 Deficiency Disorder." *Epilepsy Research* (2020): 106521.
19. Olson, Heather, et al. "Infantile Spasms In CDKL5 Deficiency Disorder Respond Poorly To First Line Treatments." *ANNALS OF NEUROLOGY*. Vol. 88. 111 RIVER ST, HOBOKEN 07030-5774, NJ USA: WILEY, 2020.
20. Pestana-Knight, Elia M., et al. "Ganaxolone Significantly Reduces Major Motor Seizures Associated With CDKL5 Deficiency Disorder: A Randomized, Double-Blind, Placebo-Controlled Phase 3 Study (Marigold Study)."

Other references

Jakimiec, Martyna, Justyna Paprocka, and Robert Śmigiel. "CDKL5 Deficiency Disorder—A Complex Epileptic Encephalopathy." *Brain Sciences* 10.2 (2020): 107.

Katayama, Syouichi, et al. "Cyclin-Dependent Kinase-Like 5 (CDKL5): Possible Cellular Signalling Targets and Involvement in CDKL5 Deficiency Disorder." *Neural Plasticity* 2020 (2020).

Benke, Tim A., and Peter C. Kind. "Proof-of-concept for a gene replacement approach to CDKL5 deficiency disorder." *Brain* 143.3 (2020): 716-718.