The 2nd International meeting of the CDKL5 Associations
Bologna, April 25th - 27th

The 2nd International meeting of the CDKL5 Associations was held from April 25th - 27th in Bologna, in the historic lecture theatre of the medical school. The meeting was organised by the Italian CDKL5 Association with support from the Association l'albero di Greta and CDKL5UK.

The first 2 days were devoted to presentations on the genetics, cell biology and clinical aspects of the CDKL5 disorder. The 3rd and final day of the meeting gave families, carers and therapists the chance to discuss various therapies that have been used for affected children.

This report is a summary of the presentations given. I have tried to present the scientific content of the meeting in hopefully a readable fashion. Inevitably there will be some parts which may not be clear (I may not have fully understood myself!!) and so, if there are any queries please don't hesitate to contact me.

Genetics

In France, they have tested over 1000 girls and boys for CDKL5. There are 3 main testing centres and just over 50 cases have been identified so far. Genetic testing is based on widely used techniques, namely Sanger Sequencing (sequence mutations) and MLPA (deletions or duplications) – although exons 1a, 1b and 16b are not included in their testing.

Specific mutations affecting the gene after exon 18 were discussed. Although X-inactivation is said to be a random event, there is an associated gene, XIST, involved at least in the initiation of the process. Amongst the various mutations encountered, it was suggested that when the CDKL5 gene is part of a large deletion on an X chromosome then that particular X chromosome may be the one that is inactivated - consequently these mutations may be silent. On the other hand, mutations that involve large translocations from an autosomal chromosome (1 to 22) onto a particular X chromosome may result in the inactivation of the other "normal" X chromosome. These mutations can therefore affect the phenotype.

As well as France, further reviews were presented from Italy (over 70 cases of CDKL5 identified), Greece (about 4 cases) and Spain (about 20 cases).

There was a good discussion about mosaicism as a cause of a CDKL5 disorder (for mosaicism, please see http://www.supporting-cdkl5.co.uk/the-genetics-of-cdkl5.php). There are a number of case reports of somatic mosaicism in the literature. Somatic mosaicism arises when a mutation occurs in a single cell after fertilisation of the egg. All subsequent daughter cells that arise from that cell will also carry the mutation while the remaining cell lines will be normal. The individual will therefore have 2 somatic cell populations – one population of cells with the mutation and the remaining population without.

Cell Biology

Cortical function in the brain represents a balance between excitatory and inhibitory inputs, and the CDKL5 disorder may be associated with a defective balance that might also underlie autism and epilepsy. Cell signalling (also known as signalling pathways) are the many complex processes that control and co-ordinate the different activities of cells. Studies from a knockout mouse model suggest that Akt-GSK3-beta signalling - a pathway containing a class of proteins involved amongst other things in the regulation of cell growth, proliferation, cell loss and survival - appears to be a target of Cdkl5 and this might indicate possible future therapeutic targets.

The CDKL5 protein is thought to have an important role in the function of dendritic spines (please see http://www.supporting-cdkl5.co.uk/the-cdkl5-protein.php) and a number of presentations focussed on this. A study in mice on the dynamics of dendritic spines suggested that in the normal situation a natural decrease in spine density is observed in early life and that this appears to be worse in Cdkl5 knockout mice. A possible reason for this is that the dynamics of dendritic spine density is a balance between dendritic spine gain and loss and there appears to be relatively more dendritic spine loss in knockout mice. Furthermore, spine loss appears to be reversed through another signalling pathway - known as the Akt-mTOR-S6 pathway. It was also noted that a partial rescue of the dendritic spine loss (i.e. less spine loss) could be achieved with injections of IGF1 (Insulin-like growth factor 1) - possibly through reversing the increase in spine loss seen in knockout mice.

Receptors are also an important part of the signalling pathway process and the N-methyl-D-aspartate (NMDA) receptor regulates the localisation of the CDKL5 protein. NMDA stimulation increases localisation of the CDKL5 protein from the spine to the dendrite.

Some relatively newer research was presented on the role of another receptor – the SRB1 receptor. The SRB1 receptor belongs to a family of receptors that have a role in scavenging – the removal of foreign or waste material - and they clearly have an important function. The SRB1 receptor is specifically involved in the processing of cholesterol via molecules called lipoproteins - you might recall the old story about High Density Lipoproteins (HDL's – the good guys) and Low Density Lipoproteins (LDL's – the bad guys). A study found that there was a 50% reduction of SRB1 receptors in CDKL5 fibroblasts (a type of cell). In addition, there is another pathway involving a "transcription factor" called NrF2. This is the main pathway for protecting against the cellular effects of oxidative stress (a process that is also being investigated in Rett syndrome). Again, levels of NrF2 seem to be reduced in CDKL5 fibroblasts while the plasma levels of something called 4-HNE (a marker of oxidative stress) are high. The bottom line of this research would appear to imply an additional mechanism in the pathophysiology of the CDKL5 disorder which again might lead to potential therapeutic options.

When a nerve cell is "resting", there is a very small negative charge inside the cell. When the nerve cell transmits a signal, there is an influx of positively charged ions – usually sodium or calcium – into the cell - a physiological event known as "depolarisation." A study was presented suggesting that depolarisation is involved in the regulation of the CDKL5 protein, that is, its production and subsequent removal. Firstly, there is a localised process possibly involving the local synthesis of the protein, and then a second pathway whereby the CDKL5 protein becomes dephosphorylated leading to its degradation. These are therefore possible pathways involved in local regulation of the CDKL5 protein in the neuron through depolarisation.

There are a family of proteins called cyclin-dependent kinases (CDK's) that are involved in the cell cycle, that is, the division of a parent cell into 2 daughter cells. It now appears that cyclin-dependent kinase-like 5 (CDKL5 - that's us!) also has a role in the cell cycle through the function of the midbody. What is a midbody - I hear you ask? Well, the midbody is the structure that connects 2 cells just before they finally separate into 2 daughter cells at the end of cell division. Midbody defects can cause a number of cellular abnormalities although I don't think these have been reported in relation to CDKL5. The role of the midbody in neurons is less well understood.

Final note - every piece of research outlined here represents a very small part of a big jigsaw puzzle that is the control and function of the CDKL5 protein we are trying to understand.

Clinical

Encephalopathy is a general term that denotes a disease that affects the function or structure of the brain. Epileptic Encephalopathy in relation to a condition such as CDKL5 is defined as an encephalopathy above and beyond what might be expected from the underlying pathology, and this was discussed. It appeared that this term was being used to refer to the nature and severity of the epilepsy rather that to other non-epileptic effects – such as on cognitive function - although it was a little confusing. There is another view that suggests we should use the term "developmental encephalopathy" to denote that what we see might purely be the result of the underlying pathology.

At some point, a discussion occurred regarding how CDKL5 should be referred to. There are obviously some individuals who feel that CDKL5 should still be considered a variant of Rett or Rett-like. This view appears to be based on some of the similarities that exist in the phenotypes of the 2 conditions despite the fact that Fehr et al (2012) clearly showed that less than a third of children with a CDKL5 disorder satisfy the revised criteria for the diagnosis of Rett variants (Neul et al 2010)

There were 2 presentations on the problems of sleep and breathing disorders. I suspect a lot of bleary-eyed mums and dads were rather hoping for some answers here, particularly in relation to managing sleep disturbances. Unfortunately, this was not to be…sigh!… Sleep studies have shown various disturbances in the pattern of sleep in children with CDKL5. The studies are small and disturbances can occur in the absence of epileptic activity and appear more likely to be related to an abnormality of the REM sleep cycle. This theory may also have been corroborated by studies on mouse models. Sleep hygiene seems to be the "official" advice – although we are sure that many parents are already "hygiened-out" and don't feel that they have anywhere else to go - apart from perhaps to the bottle!

Therapy

A review of gene therapy in mouse models of Rett syndrome was presented. Many mouse models have been established and gene therapy has been developed and applied to these models. The mechanism by which the gene is introduced into the brain (vector) is the adeno-associated virus (AAV) which is not currently known to cause disease. Therapy improves most aspects of locomotor function – except gait. Although the MeCP2 gene is contained in many different tissue types, deficits in the central nervous system are the most important in terms of being symptomatic compared to those in peripheral (heart, kidney, lung etc..) tissues.

On the 3rd and final day, a number of parents and/or their therapists gave talks on various types of therapy they had found useful for their children. These included hydrotherapy, spider therapy, pranotherapy and cranio-sacral therapy.

Overall, the meeting was attended by more than a hundred individuals. There were about 48 families who attended. Most were form Italy but others had made the journey from Germany, Spain, Holland, Slovakia, Austria, Canada and the UK. The remainder consisted of researchers, clinicians, students and therapists. Again, as in Maastricht, the concept of mixing families and researchers/clinicians worked well although it was a little disappointing that more clinical research was not available for presentation. CDKL5UK emphasised its commitment to continue raising the profile of CDKL5UK and of the CDKL5 Disorder in the UK and around Europe, and to remain committed to the development of a European CDKL5 Association to support families all over Europe and for jointly funded research.

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