

PRION 2017
Deciphering Neurodegenerative Disorders
Edinburgh 23-26.05.17

PRION 2017 was the latest of the annual international Prion Disease Conferences and, this year, took place in Edinburgh, Scotland, over 4 days from 23rd to 26th May 2017.

OVERVIEW

These meetings are attended by people from many countries and many different disciplines such as: protein chemistry, genetics, clinical neurology, epidemiology, public health and agriculture/animal medicine. This year, there were around 350 attendees and, most importantly, members of the CJD International Alliance were there to present the human side of the story. This presentation from the patient/family point of view is always greatly appreciated by the researchers and helps to give additional motivation to their work in understanding these diseases. Those who are personally affected by CJD should take heart that there is such a concerted international effort in this difficult area.

The meeting's subtitle reflected the growing interest in the relationship between prion diseases (including CJD) and other, more common, illnesses such as Alzheimer's Disease, Parkinson's Disease and Motor Neuron Disease. Whatever the differences between these diseases (in why they occur and how they affect people), they all have a particular kind of disease feature called 'neurodegeneration'. This is difficult to define succinctly but, in essence, in a neurodegenerative disease, particular groups of neurons (nerve cells) dysfunction and die associated with the tissue deposition of abnormally folded protein (in the case of prion disease, it is the prion protein). Researchers into these different diseases are, increasingly, looking to see how their knowledge may be helpful to each other.

To give a broad overview of the topics covered, the meeting programme was divided into these main areas:

- Mechanisms of Protein Misfolding
- Mechanisms of Neurodegeneration
- Current concerns in Prion Disease
- Disease Transmission and Pathogenesis
- Clinical Aspects of Prion Disease
- Diagnostics/Therapeutics

The meeting opened with an address by the Chief Scientist from the Scottish Government Health Directorates, followed by two detailed reviews what we know of population, public health, cellular and molecular aspects of prion diseases. These were followed by Sally Magnusson's moving personal account of how dementia affected her mother and how music had improved her quality of her mother's life during that time.

It is impossible to give details of all the meeting presentations and, naturally, different attendees would probably select different ones as most important-reflecting their particular research interests. What follows is a selective summary, with general comments more than specific detail, designed to give an overall flavour of the conference and, hopefully, to highlight those areas of most interest to families.

DISEASE PROCESS

There were presentations of research into the underlying mechanisms of prion and other neurodegenerative diseases. Five broad aspects stood out: firstly, the importance of the

synapse in disease, secondly, the selective involvement of neurons, the way disease spreads in the brain and the misfolding of proteins. Interesting and important progress is being made in all these areas.

- Neurons communicate with each other via special connections called synapses. It seems that the earliest phase of neurodegenerative diseases like prion disease principally affect the synapses and there is evidence that, at this early stage, the disease process could be reversible.
- One feature of neurodegenerative disease is that certain types of neurones appear to be selectively vulnerable in any given disease (at the most general level: motor neurons in Motor Neurone Disease, memory neurons in early Alzheimer's Disease etc). Within different prion diseases, one sees selective vulnerability of neurons with different distributions of pathology.
- It is known that the neurodegenerative process spreads in the brain over the disease course and, as this occurs, different symptoms arise and the disease worsens. There is a lot of work looking at whether this spreading process in, say, Alzheimer's disease is by the same mechanism as occurs in diseases like CJD.
- Proteins are very dynamic creatures, constantly being made, used, destroyed and replaced. We all make normal prion protein throughout our lives. We know that, in prion disease, abnormally folded prion protein can somehow convert normal prion protein into an abnormal form leading to accumulation of abnormal folded protein. The original abnormality could be introduced from outside (as in transmitted, infectious, forms of disease). However, in apparently spontaneous diseases, such as sporadic CJD, the original abnormality could be a simple, random, mistake in protein manufacture. These mistakes do occur, but there are 'quality control' mechanisms in cells that should deal with these errors. Why such 'quality control' systems sometimes fail or whether these 'quality control' processes themselves could have bad effects, are interesting research areas.
- Although neurons are the cells that are most relevant in the cause of symptoms, there are other cells in the brain that could well be important in disease processes. There has been a lot of developing interest in the potential role of these non-neuronal cells.

CHRONIC WASTING DISEASE (CWD)

This is an important area of animal research in North America but now with direct importance for Scandinavia, with the reports of cases in Norway, and potential importance for other areas of Europe.

The details of the Norwegian cases were presented with two apparently separate geographical areas being affected. There is a plan to attempt elimination of the disease in one of these affected areas.

Experimental details were presented illustrating how easily CWD spreads from animal to animal including by contact with ordinary body secretions, environmental contamination and with evidence of maternal transmission (from mother to unborn offspring).

Experimental evidence of successful transmission of CWD to primates (macaques) was presented.

Naturally, there is continuing concern as to whether CWD could transmit to humans. There is currently, no evidence that this has occurred. Human disease surveillance systems are looking actively to see if any such cases ever occur.

OTHER NEURODEGENERATIVE DISEASE

There were a number of presentations on diseases other than prion disease and the potential relationship with prion disease.

As selective examples:

- How alpha-synuclein (a key protein in the disease process of Parkinson's Disease) interacts with prion protein and might affect the prion disease process.
- Understanding the role of a-beta (a key protein in Alzheimer's Disease, AD) in AD.
- How normal prion protein might be relevant in AD.
- How studies of iatrogenic CJD (human growth hormone and dura mater related) have produced evidence of transmission of a-beta protein abnormality alongside the transmission of prion disease. However, it must be stressed, there is, as of yet, no evidence that AD itself has been transmitted.

CURRENT CONCERNS IN VARIANT CJD

We know that exposure to BSE infection in food can result in subclinical infection in people (infected but with no evidence of clinical disease). There are several questions surrounding this especially: how many people are subclinically infected?, are they capable of transmitting disease to others? And will they become clinically ill with variant CJD (vCJD) at some point? Two presentations addressed these questions on the basis of a recent UK study. In subclinical BSE/vCJD, tissues like the appendix may show abnormal prion protein deposition. Previous UK appendix tissue studies have suggested that 1:2000-1:4000 of the population are infected. However, despite this, clinical cases of vCJD have virtually disappeared and there have been very few instances of human-to-human transmission and none recently. The recent appendix study compared results from a time presumed to pre-date significant BSE cases, a time during the known BSE exposure and a time post-dating the presumed BSE exposure. The unexpected results were that positive samples were found in all 3 periods. Either the BSE exposure period was not as presumed or there may be some explanation for the positive results other than dietary BSE exposure.

GENETICS

There are always genetic aspects to any disease-varying from minor susceptibility effects, through major susceptibility effects to direct causation (as in inherited, genetic disease). The direct role of prion protein gene mutation in genetic prion disease is clear. The Codon-129 variation in the prion protein gene is also well understood as having a notable effect in susceptibility and clinical outcome in prion diseases. However, it is likely that there are other genetic influences at work in prion diseases-even if relatively weak. Some other genetic factors have been identified in the past for both sporadic and variant CJD. In recent times, there have been major advances in genetic technology that allow searching through huge numbers of genes to try to identify those that might have some contributory effects, even if small. One presentation described the results from a very big and detailed study that had identified some possible new gene influences. One point of such work is that, if relevant genes can be identified, it is possible to explore what these genes do and thereby gain further insights into the mechanisms of disease.

DIAGNOSTICS

There is no doubt that major improvements in diagnosis of prion disease have taken place over the years. However, there is continued research into easier, more reliable and simpler diagnostic tests. Three presentations are worthy of note:

- A new blood test has been developed that is very sensitive and specific for vCJD (variant CJD). Importantly, in this study, the test detected abnormality in some blood samples taken in a pre-clinical (subclinically infected) phase of the illness. This is the first time this has been shown.

- Work on diagnosing CJD on skin biopsy was presented. It was also shown that potential infectivity could be found in such skin samples, but only using specific experimental techniques. It should be emphasised that there is no evidence that these diseases can be naturally transmitted by ordinary, even intimate, human contact.
- An interesting presentation described a technique for detecting prion disease using fruit flies. This may well be of potential utility and does not have the ethical, time or expense problems associated with the use of larger animals.

TREATMENT

Many of the above topics could have implications for possible therapies. However, there were two specific presentations of possible therapies.

- The use of types of abnormal, but harmless, prion protein that might 'block' the effect of disease-related abnormal prion protein.
- The use of antibodies to normal prion protein in order to stop the prion protein conversion disease process.

CLOSING TALKS

The meeting closed with two talks. The first reviewed abnormalities of the neuronal synapses in neurodegenerative disease, especially in AD. The second reviewed the methods used to model neurodegenerative disease in experimental work, highlighting the utility of using cultured cells in the laboratory.

Other sessions of interest:

Cross-species transmission of CWD prions. Dr. Christina Sigurdson presented an update on her group's work characterising interactions between prion particles from different species. The ability of prions from animals raised for food to infect other livestock and even humans, as occurred during the BSE epidemic towards the end of the last century, remains a cause for concern. Recently a new type of prion – causing Chronic Wasting Disease – has appeared in populations of Eurasian deer and Dr. Sigurdson's group have been investigating how this prion might infect other species. They found that prions in diseased deer rely on prions in the recipient species being structurally similar for infection to take place, and went on to identify parts of the prion protein structure that might enhance infectivity or provide resistance to infection. By comparing structures, these results will prove useful in prioritising the management of at-risk livestock and other native species, and considering the further risk of humans becoming infected by eating contaminated meat such as venison or beef.

Detection of prions in the plasma of presymptomatic and symptomatic patients with variant Creutzfeldt-Jakob disease. Dr Chantal Fournier-Wirth presented results recently published (December 2016). Her team, working in Montpellier at the "Etablissement Francais du Sang" (French institution for blood) established a robust method to detect the agent responsible for BSE in blood samples. This is very important because, although the spread of BSE has been controlled by surveillance and feeding restrictions, it is estimated that millions of people were exposed to BSE prions. A major concern is that some individuals might be infected but have no symptoms. If they donate blood, they may transmit infection to others. To date, prion diseases can only be diagnosed after death, by detection of altered prion proteins in the brain. This paper is therefore very important. It not only uses a very small amount of blood as starting material (0.5mL) but also allows to detect prions in the blood before the appearance of the neurological symptoms (up to 31 months before). These findings may help clinicians and researchers in their endeavour to better diagnose and/or identify drugs for prion diseases.

Detection of Prion Seeds in Skins of Sporadic CJD Patients. Disease-causing prions are believed to be concentrated in the central nervous system in most forms of prion disease other than variant CJD. Indeed, spinal fluid and brain tissue examinations form the bedrock of diagnosis in prion disease. However, it is known that skin of infected animals such as mice, sheep and the more exotic kudu are known to harbour prions, detected by traditional methods; prions were only detected in the skin of one single human case of variant CJD, but never in sporadic CJD. With the advent of highly sensitive and specific technology known as Real-Time Quaking-Induce Conversion assay (RT-QuIC), scientists from Case Western University in Cleveland, Ohio set out to see if prion seeds can be detected in sporadic CJD patients. Not only did they manage to find prion seeds in the skin of sporadic CJD patients, but experimental mice injected with skin preparations from these patients caused the mice to come down with CJD; the traditional methods of detecting prions returned negative results. These observations raise the possibility using skin biopsy as a way of diagnosing CJD, and also the need to re-examine the distribution of prions in human tissues other than the central nervous system.

Detection of mammalian prions by PrP transgenic Drosophila. Dr Raymond Bujdoso, Cambridge University. Although variant CJD (vCJD) affected fewer than 200 people in the UK, it remains a major public health concern; studies have suggested that up to 1 in 2000 people may be silent carriers of infection. It's therefore crucial to have a rapid method to detect and measure infectious prions in tissue specimens or bodily fluids, known as a bioassay. Currently, the gold standard method involves infecting mice with the material to be tested, and measuring the time it takes them to become infected. Whilst this is a very robust and effective approach, it can take months or even years. A group led by Dr Bujdoso is attempting to establish a similar bioassay in fruit flies, as they reproduce and grow quicker than mice. By replacing the fly's prion protein gene with that of a cow, they reported for the first time that they could infect flies with prions that cause Bovine Spongiform Encephalopathy (BSE) – the type of prion disease that was transmitted to humans and caused vCJD. Although this work is at an early stage, they hope to try similar experiments with variant CJD. If successful, this may be an alternative, more rapid approach to identifying infection with variant CJD in days or weeks; however, whether this is quick and efficient enough to use to widely screen blood samples remains to be seen.

Muskelin protein in prion disease. Dr. Krasemann's team investigated the role of the protein muskelin in prion disease. Muskelin is what is called an "intracellular trafficking factor" this means that it works to move things around in cells. This might include movement of prions and normal prion protein. The group showed that nerve cells mice that don't have muskelin "knock out (KO) mice" display impaired transport of prion protein. Prion infection in muskelin knockout mice had a shorter incubation time. These findings highlight the importance of movement of prion proteins in prion disease and the role of protein muskelin.

A special thanks to Richard Knight and Simon Mead for their summary of this event.

