Prion 2016 Invited Lecture Abstracts

**IL-01: Molecular determinants of prions infectivity**

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Prion diseases are fatal neurodegenerative disorders caused by changes in the conformation of the physiological form of the prion protein (PrP$^{C}$$^{1}$) to alternatively folded forms denoted as prions (PrP$^{Sc}$).

Thus, prions are composed exclusively of proteinaceous aggregates of the prion protein whose tertiary and quaternary structures dictate the biological outcome of the pathology.

In an attempt to understanding the molecular mechanisms of prion formation, conversion and replication we generated and characterized a library of synthetic prions using different biochemical and biophysical parameters. The results indicate preferred conditions by which prions can be generated in vitro.

In addition, the prion protein has long been known as a copper binding protein. Copper binds to PrP$^{C}$ via histidine residues in the octapeptide repeats (OR) and the non-OR region located in the disordered N-terminal of the protein. We wondered whether copper binding plays a role in prion disease. To this end, we tested the substitution of histidine residues that may affect the prion conversion.

We created a series of mutant murine PrP (MoPrP) molecules by replacing histidine residues at OR and non-OR region. These constructs were analyzed in vitro through recombinant prion protein fibrillation assays such as PMCA, ASA, and RT-QuIC, or transfection experiments using N2a or GT1 cells.

Moreover, transgenic mice overexpressing MoPrP mutants showed clinical signs of prion disease with PK-resistant PrP in their brains. Based on these data, we could conclude that the substitution of histidine at non-OR region can enhance PrP$^{C}$-PrP$^{Sc}$ conversion process, and in particular the non-OR copper-binding site may have a critical role in this process.

**IL-02: Potential role of the environment on prion transmission: Plants, environmental surfaces and earthworms as carriers of infectious prions**

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Prion diseases are transmissible between animal-to-animal, animal-to-human and human-to-human; however, we still do not understand completely the mechanisms, factors and biological processes that control the transmission of this unique infectious agent. Some of the most prevalent and horizontally-transmissible animal prion diseases, including scrapie and CWD, have implicated environmental contamination with prions as a putative mode of transmission. Various studies have shown that infectious prions can enter the environment through saliva, feces, urine, blood or placenta from infected animals, as well as by decaying carcasses. However, it is mostly unknown which elements of the environment can act as vectors for prion transmission.
In this presentation we will describe recent experiments using PMCA to study the role of environmental prion contamination on the horizontal spreading of prion diseases. These experiments have focused on the study of the interaction of prions with plants, environmentally relevant inert surfaces and small invertebrate animals living in soil (earthworms). Our results show that plants (both leaves and roots) bind tightly to prions present in brain extracts and excreta (urine and feces) and retain even small quantities of PrPSc for long periods of time. Strikingly, ingestion of prion-contaminated leaves and roots produced disease with a 100% attack rate and an incubation period not substantially longer than feeding animals directly with scrapie brain homogenate. Furthermore, plants can uptake prions from contaminated soil and transport them to different parts of the plant tissue (stem and leaves). Similarly, prions bind tightly to a variety of environmentally-relevant surfaces, including stones, wood, metals, plastic, glass, cement, etc. Prion contaminated surfaces efficiently transmit prion disease when these materials were directly injected into the brain of animals and strikingly when the contaminated surfaces were just placed in the animal cage. Finally, studies with earthworms exposed to prion-contaminated soil show that these animals can efficiently bind, retain and geographically disperse infectious prions. Altogether, our findings demonstrate that environmental materials can efficiently bind infectious prions and act as carriers of infectivity, suggesting that they may play an important role in the horizontal transmission of the disease.

IL-03: How does recombinant prion protein become infectious? Progress in understanding the molecular basis of prion infectivity

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Transmissible Spongiform Encephalopathies (TSEs), or prion diseases, are well known for its unorthodox infectious agent, the prion. Despite decades of intensive studies, the molecular mechanism of prion infectivity remains obscure, which impedes our ability to develop effective prophylactic or therapeutic strategies against these fatal neurodegenerative disorders affecting human and animal health. We established an in vitro system that is able to synthetically generate highly infectious prions with bacterial expressed recombinant prion protein via serial protein misfolding cyclic amplification (sPMCA). Besides the recombinant prion protein, 2 cofactors are used in this system: synthetic phospholipid POPG and RNA (either total RNA from mouse liver or synthetic poly (rA) [poly-riboadenylic acid]). Recently, we also established the quantitative and highly sensitive scrapie cell assay (SCA) with CAD5 cells, which are susceptible to a wide range of prion strains. Taking advantage of these 2 assays, we compared various sPMCA products generated in the presence of identical cofactors and analyzed the role of cofactors in converting recombinant prion protein into an infectious prion. Our results support that the prion infectivity is governed by PrP conformation and cofactors also play a critical role in generating and/or maintaining the infectious prion conformation.

IL-06: Neuropathology of prion disease

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Neuropathology (considered as the tissue-based analysis of diseases of the central nervous system (CNS)) has made a major contribution to knowledge of the pathogenesis of prion infections in the CNS, mechanisms of prion-induced neurodegeneration and the spread and targeting of specific CNS regions by different prion stains. The timing, localization and nature of the accumulation of PrPSc in the CNS has widened the concept of strain properties of prions and facilitated the exploration of prion strains in human diseases, particularly
sporadic Creutzfeldt-Jakob disease (sCJD) and vCJD. Neuropathology also plays a key role in the diagnosis of prion infections in humans and animals, especially when combined with Western blotting for the detection and characterization of PrPSc isoforms in the CNS, allowing the identification of novel diseases such as variant Creutzfeldt-Jakob disease (vCJD) and protease-sensitive prionopathy.

Knowledge on the peripheral and CNS pathogenesis of prions following experimental prion infection by a range of routes (including intracerebral, oral and intravenous routes) has prompted exploration of peripheral pathogenesis in acquired human prion diseases, particularly in vCJD, where the evidence of PrPSc accumulation in lymphoid tissues (spleen, tonsil, lymph nodes, Peyer’s patches) has allowed the identification of asymptomatic infections following iatrogenic exposure to vCJD prions though non-leukodepleted red blood cell transfusion and treatment with large doses of UK plasma. More recently, the identification of disease-associated prion protein accumulation in germinal centers within the appendix has been used to estimate the prevalence of asymptomatic vCJD infection in the UK, and its occurrence in different genetic subgroups as defined by the polymorphism at codon 129 in the human prion protein gene (PRNP).

The second most common acquired form of human prion disease in the UK is iatrogenic CJD in recipients of human pituitary-derived growth hormone (iCJD-hGH), cases of which are still occurring after the first case was identified in 1985. We have explored the neuropathological phenotype and peripheral pathogenesis of the largest series of iCJD-hGH cases in the UK, involving detailed biochemical and histological analysis of prion-related pathology and co-pathology, genetic analysis and in vitro assessment of PrPSc seeding activity. These findings are compared with those in the brains of other UK recipients of hGH who did not develop iCJD, but who died from other causes, and cases of iCJD occurring in human dura mater graft recipients.

**IL-07: Iatrogenic transmission of Creutzfeldt-Jakob disease**

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Creutzfeldt-Jakob disease (CJD) is iatrogenically transmissible via dura mater grafts, growth hormone administration, neurosurgical instruments, corneal grafts and stereotactic intracranial electrodes. Iatrogenic transmission through neurosurgery had been reported in only 4 cases, and transmission due to occupational exposure had not been recognized. However, recent our study has identified 2 CJD cases, previously thought to represent sporadic CJD, actually represented acquired CJD in a neurosurgeon and in a patient with a medical history of neurosurgery without dural grafting. In addition, the Japanese CJD Surveillance registry listed 6 of 760 CJD patients who had undergone neurosurgery after the onset but before the diagnosis of CJD during the period 1999 to 2008. Although none of the individuals exposed to possibly contaminated instruments has developed CJD to date, the ensemble of these observations suggests that the potential risk of iatrogenic transmission via neurosurgical procedures may be greater than is presently appreciated. To eradicate iatrogenic CJD transmission, further investigation of acquired CJD cases and their routes of infection will be needed in the future.
IL-08: Revisiting supersaturation as a factor determining amyloid fibrillation

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Amyloid fibrils involved in various diseases including prion diseases are formed by a nucleation-growth mechanism, similar to the crystallization of solutes from solution. To study amyloid fibrils, we developed several types of unique techniques. First, to visualize amyloid fibrils, we combined total internal reflection fluorescence microscopy (TIRFM) with amyloid-specific thioflavin T fluorescence.\textsuperscript{1} With this approach, we succeeded in observing the growth of amyloid fibrils in real-time at a single fibrillar level for various amyloidogenic proteins including $\alpha\beta$. Second, we showed that ultrasonication is one of the best means of accelerating amyloid nucleation and thus the formation of fibrils.\textsuperscript{2,3} By combining a water bath-type ultrasonicator and a microplate reader, we constructed a HAN-dai Amyloid Burst Inducer (HANABI), which enables a high-throughput analysis of ultrasonication-forced amyloid formation of proteins.\textsuperscript{4} Third, calorimetry, one of the most powerful methods used to study the thermodynamic properties of globular proteins, has not played a significant role in understanding protein aggregation. We succeeded with $\beta2$-microglobulin in direct heat measurements of the formation of amyloid fibrils using isothermal titration calorimeter.\textsuperscript{5} Our results with various unique approaches indicate that the solutions of denatured proteins are often supersaturated above the solubility limit and ultrasonic agitations release the supersaturation effectively, excluding solvated monomers to form fibrils. We suggest that amyloid fibrils and amorphous aggregates are similar to the crystals and glasses of solutes, respectively, and supersaturation is required to form crystal-like amyloid fibrils. We propose a general view of how the structures of protein and peptide precipitates vary dramatically from single crystals to amyloid fibrils and amorphous aggregates, in which "solubility" and "supersaturation" play critical roles.\textsuperscript{3,6,7}

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IL-12: Activation state of glial cells in prion diseases

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Activation of glial cells is one of the hallmarks in prion diseases. There is a line of evidence that shows microglial activation have both detrimental and beneficial effects in prion diseases, whereas little is known about role of astrocyte activation. To characterize the activation state of microglia and astrocytes and to speculate roles of activated glial cells in prion diseases, we performed transcriptome analysis of glial cells isolated form brains of mice infected with prions. Mouse brains were collected from the early (60 dpi), middle (90 dpi), late (120 dpi), and the terminal stage of the disease (145 dpi), and microglia and astrocytes were isolated using magnetic activated cell sorting (MACS) with anti-CD11b and anti-astrocyte cell surface antigen-2 antibody, respectively. Comprehensive gene expression
of microglia and astrocytes were analyzed by next generation sequencer and following bioinformatics using Strand NGS and Ingenuity Pathway Analysis (IPA). Principal component analysis and hierarchical clustering revealed that microglia and astrocytes were differentially isolated by MACS. We selected microglia- (1,433 genes) and astrocyte-enriched genes (2,324 genes) that showed higher expression in microglia than astrocytes and vice versa, respectively. Among the microglia- and astrocyte-enriched genes, products of 103 and 246 genes, respectively, were expected to be secreted from cells, and we further used these gene sets for secretome analysis. Gene expression profiles of microglia-enriched secretory proteins suggested that microglia show tendency to support cell survival from the early (60 dpi) to the late stage of the disease (120 dpi). In addition, the gene expression also suggests that microglia may gradually exert cytoxic function from the middle stage of the disease (90 dpi). In contrast to microglia, gene expression profiles of astrocyte-enriched secretory proteins suggest that astrocytes may have cell protective role through the course of the infection. These tendencies appeared to be pronounced when secretome analysis was focused on functions on development of neurons and differentiation of nervous system using IPA. The cell-type specific gene expression profiles of microglia and astrocytes are invaluable information on further analysis of glia-to-glia and neuron-to-glia communications in prion diseases.

**IL-13: Transmission of prions to non human-primates: Implications for human populations**

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Prion diseases are the unique neurodegenerative proteinopathies reputed to be transmissible under field conditions since decades. The transmission of Bovine Spongiform Encephalopathy (BSE) to humans evidenced that an animal prion disease might be zoonotic under appropriate conditions. Contrarily, in the absence of obvious (epidemiological or experimental) elements supporting a transmission or genetic predispositions, prion diseases, like the other proteinopathies, are reputed to occur spontaneously (atypical animal prion strains, sporadic CJD summing 80 % of human prion cases).

Non-human primate models provided the first evidences supporting the transmissibility of human prion strains and the zoonotic potential of BSE. Among them, cynomolgus macaques brought major information for BSE risk assessment for human health, according to their phylogenetic proximity to humans and extended lifetime. We used this model to assess the risk of primary (oral) and secondary (transfusional) risk of BSE, and also the zoonotic potential of other animal prion diseases from bovine, ovine and cervid origins even after very long silent incubation periods.

We recently observed the direct transmission of a natural classical scrapie isolate to macaque after a 10-year silent incubation period, with features similar to some reported for human cases of sporadic CJD, albeit requiring fourfold longer incubation than BSE. Scrapie, as recently evoked in humanized mice, is the third potentially zoonotic prion disease (with BSE and L-type BSE), thus questioning the origin of human sporadic cases. We also observed hidden prions transmitted by blood transfusion in primate which escape to the classical diagnostic methods and extend the field of healthy carriers. We will present an updated panorama of our different long-term transmission studies and discuss the implications on risk assessment of animal prion diseases for human health and of the status of healthy carrier.

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IL-14: Variant CJD

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The primary epidemic of variant Creutzfeldt-Jakob disease (vCJD) may be nearly over. There have been no deaths from vCJD in the UK since 2013 and none worldwide since 2014. The mismatch between the observed number of cases and the estimated levels of oral exposure of human populations to BSE prions, particularly in the UK, is unexplained, but most likely relates to a significant species barrier between bovines and humans. There have been 4 cases of vCJD born after 1989 in continental Europe but none born after this date in the UK, suggesting that the measures introduced in the UK in 1989 to reduce human exposure to the BSE agent may have been effective, despite not being fully enforced.

There have been 4 instances of secondary transmission of vCJD through blood transfusion identified to date in the ongoing UK TMER study, but none reported since 2007. Detailed analysis of transfusion history in vCJD cases indicates that only a small number of such cases, if any, could have been missed by epidemiological surveillance. Transfusion transmission is a relatively efficient route of infection: there has been transmission of infection in 4/33 recipients who survived 5 y or more following transfusion from a donor who later developed vCJD, and laboratory transmission studies do not indicate an adaption of the agent after secondary transmission. These observations are difficult to reconcile with prevalence studies in the UK which have led to estimates that 1/2000 of the general population may be infected with vCJD. It is possible that measures introduced to minimise the risk of transfusion transmission, including leucodepletion of blood components, may have contributed to the limited number of transfusion transmitted cases observed.

There is, to date, no evidence of secondary transmission of vCJD via contaminated surgical instruments, via organ or tissue transplant or vertically from mother to child, but the period of observation is too short to exclude the possibility that cases linked to these potential routes of transmission may be observed in the future. There are also concerns about further outbreaks of vCJD occurring in individuals with different genetic backgrounds, but this has not been observed to date, and it is now 22 y since the clinical onset in the first case of vCJD identified in the UK.
**IL-15: Genetic prion diseases**

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Human prion diseases are fatal rare degenerative protein misfolding diseases of the nervous system with one to two affected patients in one million per year worldwide. Most cases are sporadic, 10 to 15% of human prion disease cases are genetic, caused by a mutation in the PRNP at the short arm of chromosome 20. So far, more than 30 mutations in the PRNP are known. Mutations in the PRNP lead to three groups of genetic prion diseases. Well known and characterized are the D178N mutation of PRNP in combination with the methionine allele at codon 129, called fatal familial insomnia (FFI), and the P102L mutation, called Gerstmann-Sträussler-Scheincker-syndrome (GSS). The third group of genetic TSEs is very heterogeneous and summarized as genetic CJD (gCJD).

Genetic Creutzfeldt-Jakob-diseases (gCJD) might present a similar clinical phenotype like sporadic CJD (sCJD) and no tests have been identified so far to distinguish the sporadic and genetic forms, if no genetic test (PRNP analysis) is available for various reasons. One of the important caveats is a negative family history for prion diseases. The clinical presentation in genetic CJD might vary even within the same family and the reasons for different age at onset and distinct clinical pathological phenotype are not known. Recently, the question of penetrance of certain mutations has been discussed. The identification of risk factors and modifiers of the disease are important to understand disease pathogenesis and potentials for therapeutic interventions. In absence of a positive family history only the wide genetic testing of all CJD patients allows the diagnosis of genetic disorder.

**IL-16: Biomarkers for prion disease**

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The clinical diagnosis of Creutzfeldt–Jakob disease (CJD) and other rarer phenotypic variants of human prion disease have become more frequent and more challenging. An aging population, the improved characterization of prion disease as a heterogeneous disorder covering a wider phenotypic spectrum than previously recognized, and the identification of potentially treatable disorders that manifest as rapidly progressive dementia, have all contributed to this change. The diagnostic approach of patients with suspected CJD relies on clinical features, EEG and cerebral MRI findings and the results of assays measuring protein markers in the cerebrospinal fluid (CSF) or other tissues. Protein 14-3-3 detection by western blotting is the most widely explored of these assays and the only one included in current diagnostic criteria; yet it is also the most controversial in terms of inter-laboratory findings due to the high variability of the reported specificity of the test. Among the other CSF proteins proposed as surrogate biomarker of sporadic CJD, tau protein has attracted most of the attention since total-tau (T-tau) was found comparable or even superior to 14-3-3 protein as a marker in the diagnosis of CJD. Furthermore, given that CSF levels of phosphorylated tau (P-tau) are increased in Alzheimer disease (AD) but generally not in other progressive neurologic diseases, the T-tau to P-tau ratio has also been proposed for the differential diagnosis between CJD, AD and other dementias. At variance with assays based on surrogate protein markers, real-time quaking induced conversion (RT-QuIC) uses recombinant PrP (rPrP) as a substrate to measure the capacity of CSF (or other tissues) containing PrP\textsuperscript{Sc} to induce rPrP conversion and aggregation. In the few studies conducted to date, CSF RT-QuIC has been shown to be an accurate diagnostic test for sporadic
CJD with a good sensitivity (85-87%) and virtually full specificity. Thus, CSF RT-QuIC may represent an important advance in the development of a disease-specific premortem diagnostic test for sporadic CJD. To support this claim, however, further studies involving a higher number of patients and including the whole spectrum of disease subtypes are absolutely required. We have investigated the diagnostic utility of CSF biomarker assays (14-3-3, t-tau, p-tau, and rPrP conversion), in a large non-selected clinical population with suspected prion disease. I will discuss the data on CSF biomarkers in our CJD cohort focusing on the comparison among the different assays and taking into account the known phenotypic heterogeneity of the disease.

**IL-19: Epidemiological and clinical features of human prion diseases in Japan: Prospective 17-year surveillance**

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We analyzed the epidemiological and clinical features of patients with prion diseases registered by the Creutzfeldt-Jakob Disease (CJD) Surveillance Committee, Japan over the past 17 y since 1999. Until September 2015, we obtained information on 5,041 Japanese patients suspected as having prion diseases, and judged that 2,596 patients had prion diseases. The annual incidence rate/million of prion diseases during 1999-2013 ranged from 0.7 (1999) to 1.8 (2011/2012/2013) with an average 1.3. The 2,596 patients with prion diseases were classified into 1,999 (77.0%) with sporadic CJD (sCJD), 501 (19.3%) with genetic prion diseases, 87 (3.4%) with acquired prion diseases, including 86 cases of dura mater graft-associated CJD (dCJD) and one case of variant CJD, and 7 cases of unclassified CJD (0.3%). In sCJD, MM1 type (Parchi’s classification) is most common; among atypical sCJD cases, MM2 type appeared most common, probably related to the fact that methionine homozygosity at codon 129 polymorphism of the prion protein gene (\(PrP\)) was very common (93%) in the general Japanese population. As for iatrogenic CJD, only dCJD cases were reported in Japan and, combined with the data from previous surveillance systems, the total number of dura mater graft-associated Creutzfeldt-Jakob disease was 149, comprising the majority of worldwide dCJD patients. Most of the dCJD patients received cadaveric dura mater graft before or in 1987, and we still had onset of dCJD cases in recent years; the incubation period (i.e., duration from implantation of dura mater grafts to dCJD onset) was increasing to 13.2 ± 6.3 (mean ± SD) years (range, 1 to 30 years). Regarding genetic prion diseases, the most common \(PrP\) mutation was V180I (46.7%), followed by P102L (16.6%), E200K (14.0%), and M232R (13.6%). The distribution of the mutations was quite different from that in Europe. The V180I and M232R mutations were quite rare worldwide. Interestingly, patients with V180I or M232R rarely had a family history of prion diseases, and our recent study with large population control cohorts indicated that such missense variants have significant effects on lifetime prion disease risk. In conclusion, our data from the prospective 17-year surveillance revealed distinct features of human prion diseases in Japan: frequent and continuous occurrence of dCJD, and unique phenotypes of sCJD and genetic prion diseases related to the characteristic distribution of \(PrP\) mutations and polymorphisms in the
Japanese population, different from those in western countries.

**IL-21: Surveillance of prion diseases in Taiwan**

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**Objective:** To compare the incidence rate from 1993 to 20105 and figure out the incidence increment and characteristics of CJD in Taiwan through Surveillance of prion disease in Taiwan.

**Background:** In Taiwan, national CJD surveillance unit (CJDSU) was setup in Taiwan Neurological Society since 1997. The incidence rates of CJD were raised up after 2007 in Taiwan and also in other industrial countries. For this reason, we try to figure out the risk factors which might be connected with this phenomenon.

**Methods:** Registry and Surveillance system in Taiwan: The surveillance system was a nationwide, hospital-based case report system, which was directed by the government organization, Centers for Disease Control of Taiwan, and Taiwan Neurological society since 1997. This CJDSU system performed a national survey to all the hospitals in Taiwan. In 2007, the health government announced CJD is one of the forth statutory epidemiological diseases. All suspected cases are requested to registry on epidemiological disease system online and notify the CJDSU for consensus of diagnosis, tracing and assess differences of categorical and continuous variables between 2 period groups (before and after 2007).

**Results:** There were more than 500 cases prospectively reported to the CJDSU. Among them, 370 cases were diagnosed with CJD; with a sex ration of M/F 1:1.09. All cases were refereed as probable or possible cases except 4 cases of GSS form and 1 probable case of new-variant CJD immigrant from UK. The incidence rates were between 0.30-1.492 per million populations, and case numbers had notified double during 2008 to 2015 with incidence raised up to 1.414, which had been fall after 2014. Between two groups, the clinical features, EEG, MRI, genetic polymorphism, and CSF 14-3-3 protein had no differences, but age-onset is earlier than before.

**Conclusion:** The incidence rate of CJD in Taiwan after 2007 was extremely higher than before, probably due to the physician’s sensitivity, improved reporting system, people concerning of variant CJD cases, and the high media coverage.

**Keywords:** CJD, incidence rate, nvCJD, polymorphism, Taiwan
IL-22: Real-time quaking-induced conversion analysis for the diagnosis of sporadic Creutzfeldt-Jakob disease in Korea

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The 14-3-3 protein is increased in the cerebrospinal fluid (CSF) of Creutzfeldt-Jakob disease (CJD) patients and has been thus used as a clinical biomarker for the pre-mortem diagnosis of human prion diseases. Nonetheless, the specificity of 14-3-3 protein is less reliable for CJD diagnosis. The newly developed assays including real-time quaking-induced conversion (RT-QuIC) made it possible to detect PrP<sup>Sc</sup>-like abnormal prion isoform with a high sensitivity in animal and human specimens that might have a minute amount of PrP<sup>Sc</sup> by in vitro prion replication. In this study, we performed a highly sensitive RT-QuIC assay using recombinant human PrP for the detection of PrP<sup>Sc</sup> in CSF of 100 sporadic CJD (sCJD) patients in Korea. By analyzing CSF samples of 100 patients with sCJD and of 190 non-CJD patients based on the expression levels of 14-3-3, total tau and RT-QuIC assay, the positivity of RT-QuIC analysis was observed in 77 of 100 CSF samples (sensitivity 77%) but no positive in the non-CJD patients. The sensitivity of RT-QuIC in this study was similar to that in some previous reports and the specificity of RT-QuIC was higher than that of 14-3-3 in CSF, suggesting that RT-QuIC analysis can complement the weakness of the specificity of 14-3-3 for the diagnosis of sCJD. These results indicate that RT-QuIC might be of great use for rapid and specific diagnosis of sCJD and suggest a practicable novel method for the ante-mortem diagnosis of human prion diseases.

This research was supported by the National Research Foundation of Korea Grant Funded by the Korean Government (NRF-2011K3A1A1003362) and by Hallym University Specialization Fund (HRF-S-41).

Keywords. Creutzfeldt-Jakob disease, cerebrospinal fluid, RT-QuIC, 14-3-3, total tau

IL-23: Therapeutic approaches to prion infection and disease

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Prions are transmissible agents which cause lethal neurodegenerative diseases of humans and other animals. They appear devoid of nucleic acid and composed of assemblies of misfolded host-encoded cellular prion protein which can faithfully propagate in vivo. Recent advances suggest that prions themselves may not be directly neurotoxic, but rather their propagation leads to production of toxic species which may be uncoupled from infectivity. The commonest human prion disease, sporadic Creutzfeldt-Jakob disease (sCJD), is an invariably fatal sub-acute dementia with a mean clinical duration of 4 months and no therapeutic options. Prion strains consist of a population or cloud of species which may evolve under drug selection leading to rapid development of resistance to drugs targeting disease-associated PrP assemblies. Targeting normal cellular PrP, rather than prions themselves, has proved therapeutically effective in animal models by removing or sequestering the substrate for prion propagation and passive immunotherapy with anti-PrP monoclonal antibodies has shown therapeutic effects in several animal models. The MRC Prion Unit has developed humanised anti-PrP monoclonal antibodies and a clinical candidate developed and subjected to safety and other testing with a view to clinical trials in patients with sCJD. Emerging evidence suggests that cellular PrP has a direct role in mediating some aspects of neurotoxicity in
Alzheimer disease which may allow common therapeutic approaches.

IL-24: Lessons from recent outcomes of clinical trials and therapeutic studies

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Recently tremendous efforts have made it feasible to conduct large-scale clinical trials with a structured framework, but few meaningful outcomes have benefited CJD patients. In this paper, the issues to be solved are discussed for achieving significantly beneficial intervention against prion disease.

One of the main reasons clinical trials commonly fail in terms of survival is delayed intervention. In prion-infected animals, the more delayed the intervention, the less effective anti-prion compounds are at prolonging survival. The most opportune time for therapeutic intervention of prion diseases is very early in the preclinical stage, because exponentially accumulated amounts of prion almost reach a plateau in the brain prior to symptomatic disease onset. Consequently, suitable preclinical diagnostic measurements are required for preemptive intervention. Recent epoch-making inventions such as PMCA and QUIC for detecting an ultra-trace amount of PrP<sup>Sc</sup> from patients have opened new avenues for early diagnosis. However, these techniques still need to be tuned-up for diagnosing very early preclinical stages in healthy prion carriers.

Another issue is the limitation of monotherapy. Even the most beneficial compound has not been capable of halting disease progression. Limitations of monotherapy are attributable to the induction of detoxification systems and the emergence of drug-resistant variants, both of which are always observed in the monotherapy for cancers or viruses. Consequently, combination therapy using drugs with different structures and targets is also considered for treating prion diseases.

Levels of PrP<sup>Sc</sup> accumulation and infectivity are not parallel to those of neurological deterioration, suggesting that innovations in treatment strategies against PrP<sup>Sc</sup>-induced neurodegenerative processes might be as important as those for inhibiting PrP<sup>Sc</sup> accumulation. Even life-long survival with a preserved quality of life may be possible irrespective of PrP<sup>Sc</sup> levels in the brain if most effective specific treatments for the neurodegenerative processes could be introduced from very early preclinical stages.

Finally, it remains unclear whether the illness invariably occurs in those who have already been infected with prion, as this illness apparently occurs only in a certain portion of people. For example, many mutations in PRNP are linked to familial types of prion disease, although even carrying disease-linked PRNP mutations does not necessarily mean that a person will inevitably develop the disease. Consequently, it is presumed that there are other genetic and environmental factors strongly affecting disease susceptibility. Understanding these factors could be useful for therapeutic development for prion diseases.

IL-26: Logical design of a therapeutic agent for prion diseases

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We have developed a logical design system targeting the prion diseases. First, we identified the susceptible region in PrP<sup>Sc</sup> structure using NMR relaxation experiments, and various NMR experiments under the in vitro conversion conditions. Second, we developed a drug design software, 'NAGARA' which includes docking simulation, molecular dynamics simulation and quantum chemical calculation. Using NAGARA, we were able to discover the novel anti-prion lead compound and optimize it. Third, for the first time in academia, we installed various organic synthesis system including automatic robotic combinatorial synthesis system, organic synthesis system based
on GMP standards, and pharmaceutical preparation system for injection based on GMP standards. Fourth, we developed the in vivo treatment examination system for prion diseases considering administration methods as well as pharmaceutical kinetics measurements for various animals including non-human primates. Fifth, we organized the Japanese Consortium of Prion Diseases (JACOP, President: Dr. Mizusawa) for clinical trial of the candidate compounds for prion diseases.

Initial candidate would be a medical chaperone (MC). MC is the anti-prion compound optimized from the lead compound, GN8. It binds to the hot-spot of PrP\textsuperscript{C} and strongly inhibited the conformational conversion reaction from PrP\textsuperscript{C} to PrP\textsuperscript{SC}. We investigated various in vivo effects due to the administration of this compound.