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Poster Abstracts
Prion and Prion-like Diseases in Humans

HD.01: Prion mutations among patients with diverse neurodegenerative dementia in Korea

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Introduction. Genetic prion diseases, such as Creutzfeldt-Jakob disease (CJD), Gerstmann-Straussler-Scheinker disease (GSS), Fatal Familial Insomnia (FFI), are associated with the mutations of prion (PRNP) gene. More than 30 pathogenic PRNP mutations were identified. Since Alzheimer disease (AD) and prion diseases have many similarities in the neuropathological and physiological symptoms, we tested 89 Korean patients with different neurodegenerative disorders, AD, CJD, Parkinson disease (PD), Mild cognitive impairment (MCI) and Frontotemporal dementia (FTD), for prion mutations.

Materials and Methods. In total, 89 dementia patients under 60 y of age were screened for prion mutation, 45 AD patients, 8 FTD patients, 1 PD patients, 15 MCI patients, 11 patients with subjective memory impairment, 3 patients with vascular cognitive impairment, 1 CJD patients, 3 normal person (kin to the dementia patient), 1 patients with transient global amnesia and 1 patient with Hashimoto encephalopathy. The coding region (exon2) of PRNP gene was amplified by PCR, and PCR products were purified by specific kit. Both of the 5′ and 3′ bands of the PCR amplicons were sequenced.

Results. We detected three pathogenic prion mutations among 89 dementia patients. Glu200Lys was found in two patients, whom were clinically diagnosed as AD previously. Met232Arg was detected in four patients, where 2 among 4 were clinically diagnosed as MCI patients, one with AD patient and one with normal individual. Val180Ile was also reported in one clinically diagnosed AD patient.

Conclusion. Both Met232Arg and Val180Ile are associated with familial or sporadic CJD in Korea and Japan. Met232Arg might be involved in other kind of dementia too, because it was described in a Japanese patient with Dementia with Lewy bodies (DLB). Val180Ile was found in a CJD patient with Alzheimer type pathology in Japan. The effects of prion mutations seem to be heterogeneous. Hence, it might be possible that PRNP could be a potential risk factor for AD. There is another option that these patients might be CJD patients. Our findings suggest that genetic analysis could improve the differential diagnosis of dementia and the genetic testing for prion mutations should be important for dementia patients.

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HD.02: Analysis of sporadic Creutzfeldt-Jakob disease patients cerebrospinal fluid by real-time quaking-induced conversion

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Background. Creutzfeldt-Jakob disease (CJD) is caused by scrapie prion protein (PrP^{Sc}), the pathogenic form of cellular prion protein (PrP^{C}), which is able to convert PrP^{C} into PrP^{Sc}. About 85% CJD cases occur sporadic (sCJD) Premortem patients are classified as probable sCJD cases by clinical features, electroencephalography and detection of biomarkers in cerebrospinal fluid (CSF). For a definite diagnosis brain biopsy is required. Real-time quaking-induced conversion (RT-QuIC) uses the self-propagating properties of PrP^{Sc} to amplify it up to a detectable limit, by use of recombinant prion protein (rPrP) as a substrate and intermittent automated shaking. Fluorescence detection of aggregated PrP^{Sc} monitors amplification in real-time. Three recent studies showed the potential of RT-QuIC to detect PrP^{Sc} in CSF of CJD patients.

Materials and Methods. CSF samples of 64 sCJD and 61 control patients were analyzed by RT-QuIC. All sCJD patients were classified as definite cases by neuropathological examination. Control patients were clinically or pathologically proven to have an alternative diagnosis. CSF was supplemented with working buffer including rPrP and incubated in a plate reader at 42°C for 80 h with intermittent shaking cycles. Aggregate formation was determined by measuring ThioflavinT fluorescence signal in relative fluorescence units (rfu). By statistical analysis

the best cut-off value was estimated to be 10,000 rfu and applied to determine sensitivity and specificity of RT-QuIC. To investigate differences between sCJD and control patient groups, three variables of interest were defined: relative areas under the curve (relAUCs), time to 10,000 rfu and point of maximum RT-QuIC seeding activity.

Results and Conclusion. RT-QuIC assay had a sensitivity of 81.3% and a specificity of 98.3% for diagnosis of sCJD in CSF. No influence on the RT-QuIC signal could be observed by division into age or gender groups. To analyze the influence of time from disease onset, data was grouped by the time when the lumbar puncture was performed—in early, middle or late disease stage. For late disease stages a higher RT-QuIC response was noticed and a lower response for earlier disease stages. The analysis of data by disease duration demonstrated a clear effect of higher response in patients with short disease duration followed by the middle and long disease duration. From our data we infer that RT-QuIC is an applicable and high specific method to detect PrP^{Sc} in CSF of sCJD patients. Due to its high specificity, the RT-QuIC assay can be used for diagnostic application. References can be requested.

HD.03: Proteomic and transcriptomic analysis of rats clinically affected with scrapie

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While extensive studies on the clinical and preclinical molecular pathogenesis of scrapie in mice have been completed, the direct relevance of these findings to human disease remains obscure. Molecular pathology of human prion disease is limited to clinical and post-mortem analysis. To better frame the molecular pathogenesis of prion diseases, identify new biomarkers, and better understand the variability associated with existing surrogate biomarkers of CJD, in both preclinical and clinical phases of disease, we have generated a rat prion disease by adapting mouse RML scrapie agent into the rat. In addition to providing a second rodent prion disease for comparative transcriptional analyses, the rat presents another important opportunity, the availability of sufficient amounts of cerebral spinal fluid, CSF, for biomarker discovery using proteomic approaches. In initial experiments, gene expression profiles and proteomic detection was performed on brain and CSF from rats clinically affected with our rat adapted scrapie agent. Gene expression profiles determined that rats respond to prion infection by inducing a neuroinflammatory response, consistent with prion disease responses from mouse, sheep, bovine and humans. At the level of individual genes, however, rats diverged from mouse substantially and a majority of transcripts found to be upregulated 2-fold in mice were not detected in rat adapted scrapie. Similarly, many genes upregulated 2-fold in rats were not increased in mouse scrapie. In comparative analysis of the cerebral spinal fluid from clinical rats, many of the same surrogate markers of prion disease as in humans, were measured, suggesting the relevance of rat prion disease for detection and identification of biomarkers from preclinical samples. Moreover, correlation was observed between upregulated genes in brain and enriched proteins in CSF cross-validating the data sets. We conclude that comparative studies of prion disease may be informative by identify the natural variability in the response to prion diseases. By identifying those responses which are conserved among species, the molecular basis of prion pathology can be better understood.

HD.04: A case of Gerstmann-Sträussler-Scheinker disease in Korea

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Gerstmann-Sträussler-Scheinker disease (GSS) is a type of human transmissible spongiform encephalopathy that is determined genetically. A 46-y-old woman presented with a slowly progressive ataxic gait and cognitive decline. She was alert but did not cooperate well due to severe dementia and dysarthria. High signal intensities in the cerebral cortices were evident in magnetic resonance imaging (MRI), especially in diffusion-weighted images. A prion protein gene (PRNP) analysis revealed a P102L (proline-to-leucine) mutation in codon 102. This is the first reported case of GSS (confirmed by PRNP analysis) in Korea. Distinctive MRI findings are also presented.

HD.05: Analysis of the efficiency of tau protein as multi-marker for the diagnosis of Creutzfeldt-Jakob disease in the laboratory

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The diagnostic criteria for the Creutzfeldt-Jacob disease (CJD) with electroencephalography (EEG), brain MRI and positive 14-3-3 protein detection in the cerebrospinal fluid (CSF) have been established by the World Health Organization. However, targeting the 14-3-3 protein alone on immunoblot results in low specificity. Also, the decision of weak positive for 14-3-3 protein can be sometimes false positive causing incorrect specificity. It has been used for diagnosis of Creutzfeldt-Jakob disease (CJD) as a biomarker, but a weak positive for 14-3-3 protein can sometimes represent a false positive or false-negative result, thus

leading to incorrect diagnostic result. We tried to use the tau protein as a multiple marker to improve diagnostic specificity. Total 151 CSF samples of suspected cases of CJD were investigated. The total tau (t-tau) and phosphorylated tau (p-tau) proteins were measured via ELISA. In addition, the ratio of p-tau to t-tau proteins (p/t ratio) was calculated. We found that the t-tau levels and the p/t ratio were strongly increased and decreased in the CJD group, respectively, whereas the levels of p-tau were similar in both the CJD and non-CJD groups. The combined assay of the presence of 14-3-3 protein with the level of t-tau and the p/t ratio improved the specificity of the 14-3-3 protein (47% to 76% and 87%, respectively), but reduced its sensitivity slightly (92% to 87% and 89%, respectively). The positive and negative predictive values of the p/t ratio combined with the 14-3-3 protein were very high (93% and 91%, respectively). The false positive and negative rates of the p/t ratio combined with the 14-3-3 protein were also impressive (19% and 5%, respectively). Compared with a single marker, the combination of the 14-3-3 protein and the p/t ratio clearly improved specificity of the marker to optimal levels. In conclusion, the tau proteins are useful as a biomarker when used in combination with the 14-3-3 protein for the precise diagnosis of CJD.

HD.06: RT-QuIC of olfactory neuroepithelium brushings as a potential definitive intravital diagnosis of sporadic Creutzfeldt-Jakob disease

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Background. Definitive diagnosis of sporadic Creutzfeldt-Jakob disease (sCJD) in living patients remains a challenge. Real time quaking-induced conversion (RT-QuIC) testing of cerebrospinal fluid (CSF) has allowed identification of sCJD patients with 80–90% sensitivity. However, because CJD is transmissible, untreatable and fatal, it is important to eliminate missed diagnoses. Previous work identified abnormal prion protein (PrP) in olfactory neuroepithelium of sCJD patients, prompting us to investigate whether RT-QuIC analysis of easily accessible nasal brushings might improve sCJD diagnosis.

Materials and Methods. We tested olfactory neuroepithelium brushings from sCJD and non-CJD patients using RT-QuIC, which is an ultrasensitive, multi-well plate-based fluorescence assay involving prion-seeded polymerization of recombinant PrP into amyloid fibrils.

Results. We observed strong positive RT-QuIC reactions seeded with nasal brushings from 7 of 7 probable sCJD patients, but none of 11 negative controls, providing 100% sensitivity and 100% specificity. By comparison, 6 out of 7 CSF samples from the same group of sCJD patients was RT-QuIC-positive, giving 86% sensitivity. Quantitative RT-QuIC showed that olfactory brushings contained ~105–107 prion seeds.

Conclusion. Nasal brushing-based RT-QuIC may markedly facilitate and strengthen diagnosis of sCJD. Moreover, the high levels of prion seeding activity found in these samples raises concerns about transmissible sCJD prion shedding from olfactory mucosa.

HD.07: Prion reference testing in Canada

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The Prion Laboratory Services (PLS) at the Canadian Science Centre for Human and Animal Health in Winnipeg, Manitoba is the only laboratory in Canada performing reference testing for Creutzfeldt-Jakob Disease (CJD) and other human prion diseases. Operating in a state-of-the-art facility, sample processing is completed in a Biological Safety Level 3 containment laboratory, providing a high level of protection for the operating technicians as well as the environment.

Under ISO 17025 accreditation, the PLS laboratory offers a total of four diagnostic tests: PRNP genetic sequencing from blood and tissue samples and a cerebrospinal fluid (CSF) panel consisting of three immunoassays. The panel consists of analyzing total microtubule-associated protein Tau and S100 protein quantitatively by two separate commercial sandwich ELISA kits. The third test of the panel is a Western Blot for 14-3-3 protein detection. The panel results are among the criteria used to include or exclude prion disease in the differential diagnoses. Genetic testing is performed on genomic DNA isolated from blood or brain tissue. Exon 3 of the PRNP gene is amplified, sequenced, and analyzed for previously reported mutations that may include point mutations, deletions and/or insertions known to be causative for prion disease as well as novel mutations.

The PLS is also involved in efforts to optimize, harmonise, and standardise real-time quaking induced conversion (RT-QuIC) analysis of CSF in the diagnosis of sporadic Creutzfeldt-Jakob Disease. In addition, the identification of additional biomarkers of prion and other misfolded protein diseases is under investigation by the PLS.

HD.08: MRI white matter lesions and their influence on the clinical course of sporadic CJD

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Introduction. Magnetic resonance imaging is an important diagnostic tool in Creutzfeldt-Jakob diseases. High signal changes are detected in various areas in DWI and FLAIR sequences. In addition, white matter lesions are observed in many patients. Until today their significance is not understood. In other neurodegenerative conditions, such as Alzheimer disease, there is an ongoing discussion if WML might accelerate the disease course. Here we investigate the impact of WML load on disease phenotype in sCJD patients. We were interested in this subject because of previous reports on so-called panencephalopathic CJD type,[1] which is characterized by major impairment of the white matter.

Materials and Methods. Two groups of sCJD patients were identified within the database of the National Reference Center for TSE in Germany (diagnosis: definite or probable CJD[2]). Fourty-two out of 70 patients had marked WML load on MRI scans. The WML load was quantified using two commonly used scales (Wahlund[3] and Scheltens[4]) by an experienced neuroradiologist. Both groups were characterized according to sex, codon 129 polymorphism, PrP subtype, clinical presentation, MRI- and EEG-findings typical for CJD and detectable proteins in cerebrospinal fluid such as 14-3-3 and tau.

Results. In our study we could not detect any remarkable difference in the course of sCJD patients between both groups which could certainly be linked to the presence or absence of white matter lesions. In detail: only few symptoms (e.g., paraesthesia, sleep and visual disturbances) showed variable occurrence in the different groups. As expected patients in our study group (CJD + WML) were older at disease onset than patients in the control group.

Conclusion. White matter lesions on MRI might represent a finding which is associated with older age, however, they do not modify the disease course in sCJD. Their significance is not clear and their presence is very likely to be a chance finding in CJD patients. Presence of WML, even if their total load was very high, does not rule out the diagnosis of CJD. Therefore it stays questionable if white matter lesions lead to a remarkable pathology at all.

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HD.09: Temporal degradation of prion biomarkers and relative efficacy of different storage tubes

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Keywords: prion protein; cryopreservation, temporal degradation, proteomics, storage

In applied prion proteomics research, differential regulation of proteins is one of the most promising areas of interest. However, the reproducibility of the results of quantitative proteomic experiments is often poor. As a consequence, efforts are underway to introduce reporting requirements for technical procedures in proteomic research. Cryopreservation has been considered as an efficient strategy for conserving metabolome of biological samples with some predictive negative effects.[1,2] However, storing protein extracts at different temperatures and freeze/thaw could significantly altered quantitative reproducibility of the proteome. In our study, we compared the temporal degradation of different prion biomarkers and checked the relative efficacy of normal Eppendorff tubes and Cryotubes (cryo pure tubes) over the course of Cryopreservation. The reliability of quantitative 1-DE experiments after storage of protein extracts under different conditions demonstrated different impact at -80°C. Tau expression was regulated at different time intervals but the regulation is not significant. Furthermore, posttranslational modifications are mostly not resistant to environmental shocks, but our data showed no significant decrease of p-Tau expression after 7 days of storage time. In conclusion, short-term storage conditions do not alter the quality of proteome results, however long-term studies in cerebrospinal fluid (CSF) and blood required for further verification.

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HD.10: Immune responses in rapid dementia: A comparative study on neuroinflammatory markers in CJD, AD, rpAD and MS patients

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Objective. Immunological responses in neurodegenerative disease pathogenesis such as in Alzheimer disease (AD) and prion disease have become of increasing scientific interest in the past years. A main neuropathological hallmark of both neurodegenerative diseases constitutes the extracellular deposition of amyloid fibrils of A-β (Aβ) for AD and PrP^{Sc} for Creutzfeldt-Jakob disease (CJD), leading to subsequent neurodegeneration. In these aggregational or conformational disorders it was shown that amyloid depositions co-localize with a broad variety of inflammation-related proteins (complement factors, acute phase proteins, pro-inflammatory cytokines) and activated microglia, suggesting a major influence of the brain's innate immune systeme and a chronic inflammatory process in disease pathogenesis.

Materials and Methods. Using a cytokine multiplex array we studied 17 pro- and anti-inflammatory cytokines in cerebrospinal fluid (CSF) and serum samples in patients with CJD and AD including a subgroup of rapid progressive AD (rpAD). As controls we included patients with multiple sclerosis (MS) as the most frequent chronic inflammatory CNS disease and controls.

Results. As major findings we found significantly elevated concentrations of proinflammatory cytokines (IL-6, IL-7, IL-13 and TNFα, G-CSF) in serum of patients with rpAD when compared with AD, CJD, MS and controls. G-CSF was significantly elevated in serum both of rpAD und AD patients. The immune response profile was reflected predominantly by TH2-associated cytokines (IL-6, IL-13 and G-CSF). In CSF we revealed significantly elevated levels of IL-8 und MCP in CJD (IL-8, MCP-1) und AD (MCP-1) patients.

Conclusion. Elevated levels of proinflammatory cytokines in serum of rpAD patients represent a novel finding. Our findings might reflect a systemic immune response in this group that was not presented in the same way in MS, AD or CJD as disease controls. Elevation of the chemokines IL-8 and MCP-1 in CSF in CJD and AD were partially reported before. Our findings show in two different ways an activation of the immune system in rapid dementia that was distinguishable from MS and controls. An understanding of the neuroinflammatory process in neurodegenerative dementias becomes more and more important as it might reveal new insights in the pathogenesis especially in rapid progressive forms (rpAD, CJD).

HD.11: Developing new epidemiological prion disease databases (epiCJD)

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Background. Prion disease (PrD) or Transmissible Spongiform Encephalopathy (TSE) is a group of rare diseases which affecting several mammals, including man. According to molecular profiles, such as the presentation of codon 129, those diseases show a broad of histopathological, clinical and molecular characteristics, resulting in a challenging diagnosis. Recently, an unusual association of CJD with catatonia was reported and others reports are also showing that psychiatric symptoms, such as depression, are more common that previously assumed. Recognize clinical trait are an important step to include PrD in differential diagnosis and give the most informative scenario as possible to patients's relatives in order to precede future therapies. However, illness with great heterogeneity requires standard protocols and active surveillance able to identify new features. Databases are an extraordinary approach that provides to us the possibility to catalog and monitoring a number of biological information such as genetic variation and epidemiological traits. In order to improve knowledge and better recognition of symptoms and characteristics of PrD, we are implementing the epiCJD database and an information system to collect information about these illnesses from scientific publication around the world allowing to anyone visualize reported cases through a unique website (which will be released next April).

Materials and Methods. To feed our database, we performed an active search in Pubmed bank and looked for reported cases only. Reviews and metanalysis were not considered in this search in order to avoid the occurrence of redundancies. We have cataloged epidemiological, genetic and socio-demographic data related to each reported case. A link for the original case is presented to provide a depth understanding of the cases. Users can perform searches with any data available in the database, such as the number of cases reported at a specific country or city, by clinical symptoms or a combination of different kind of information. Besides, graphics and metaanalysis will be allowed. epiCJD can be accessed through the dcjBRASIL platform in English or Portuguese. This website contains information about PrD oriented to general public and health professionals aiming to diminish the lack of information, mainly in Brazil.

Conclusion. epiCJD can be accessed by researchers, students and general public allowing them to recognize symptoms related to PrD. This approach can also contribute to surveillance of these illnesses providing to public health agencies a new way to monitor PrD around the world.

HD.12: Comparative study of the distribution of the prion protein in the squirrel monkey (*Saimiri sciureus*) following experimental challenge with variant and sporadic CJD

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Introduction. Reports suggest that the number of tissues and organs showing the presence of the abnormal prion protein (PrP^{TSE}) in variant CJD (vCJD) patients may be greater than previously thought. A limited peripheral involvement in some cases of sporadic CJD (sCJD) has also been reported. This accumulation of PrP^{TSE} outside the brain has raised concerns about the possible iatrogenic transmission risk of vCJD. The squirrel monkey (*Saimiri sciureus*) has been shown to be highly susceptible to experimental challenge with human prion disease. Neuropathological and biochemical analyses of CNS tissue have shown that sCJD and vCJD can be distinguished in the squirrel monkey and that many of the strain characteristics that define these agents are conserved after transmission. Following on from these initial studies, immunohistochemistry and western blot analysis were performed on a wide range of peripheral tissues including, lymphoreticular tissues and peripheral neural tissue to establish the full-body distribution of PrP^{TSE} in this primate animal model.

Materials and Methods. Brain homogenates from sCJD or vCJD patients were inoculated into the frontal cortex of squirrel monkeys. Animals were kept under constant clinical surveillance. At post-mortem, formalin fixed CNS tissue and a wide range of peripheral tissues were taken for immunohistochemical analysis together with frozen tissues taken for the biochemical detection of PrP^{TSE}.

Results. Immunohistochemical analysis showed no evidence of PrP^{TSE} deposition in peripheral tissues in either variant or sporadic CJD-infected animals. However, western blot assays detected PrP^{TSE} in the spleen of a proportion of the vCJD-infected animals. The PrP^{TSE} isotype resembled that detected in CNS tissue from the vCJD- infected animals and from human vCJD cases. In addition, western blot analysis detected PrP^{TSE} in the spleen of a single animal following challenge with sporadic CJD. The PrP^{TSE} type in this animal resembled that found in CNS tissue from the same animal, with a PrP^{TSE} type similar to that found in human sCJD type 1 cases.

Conclusion. This study confirms the accumulation of PrP^{TSE} in the CNS and spleen of a proportion of squirrel monkeys infected intra-cerebrally with human vCJD. Furthermore, this study extends the evidence that there may be a peripheral involvement in some cases of sCJD. PrP^{TSE} typing confirms the conservation of PrP^{TSE} type on transmission to the squirrel monkey and suggests that there are no tissue-specific adaptations in the biochemical phenotype of the agent strain following primate-to-primate transmission.

HD.13: CWD infection in the spleen of humanized transgenic mice

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Chronic wasting disease (CWD) is a widespread prion disease in free-ranging and captive cervid species in North America, and there is evidence suggesting the existence of multiple CWD strains. The susceptibility of human CNS and peripheral organs to the various CWD prion strains remains largely unclear. Current literature suggests that the classical CWD strain is unlikely to infect human brain, but the potential for peripheral infection by CWD in humans is unknown. We detected protease-resistant PrP^{Sc} in the spleens of a few humanized transgenic mice that were intracerebrally inoculated with natural CWD isolates, but PrP^{Sc} was not detected in the brains of any of the CWD-inoculated mice. Our ongoing bioassays in humanized Tg mice indicate that intracerebral challenge with such PrP^{Sc}-positive humanized mouse spleen already led to prion disease in most animals. These results indicate that the CWD prion may have the potential to infect human peripheral lymphoid tissues.

HD.14: Nanopore analysis demonstrates binding of recombinant T194A bovine PrP^{C} by PrP^{Sc} specific antibodies: Potential implications for immunotherapy of familial prion diseases

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Transmissible spongiform encephalopathies (TSE's) are fatal neurodegenerative diseases based on the misfolding of a self-protein (PrP^{C}) into an infectious, pathological conformation (PrP^{Sc}). There is proof-of-principle evidence that prion immunotherapy is possible but this is tempered with concerns of potential dangers associated with induction of immune responses to a widely expressed self-protein. By targeting epitopes which are specifically exposed upon misfolding our group has developed a vaccine that generates antibodies which discriminates PrP^{Sc} from PrP^{C}. In a number of species we have demonstrated that these PrP^{Sc}-specific IgG antibodies do not bind wild type PrP^{C}. Here we consider interaction of a PrP^{Sc} specific polyclonal antibody SN6b with prion protein isoforms associated with spontaneous prion disease. Using nanopore analysis under non-denaturing conditions, we observed binding of the SN6b to recombinant bovine prion protein with mutation T194A [bPrP(T194A)], which is a homolog of the human T183A mutation of PrP^{C} associated with early onset of familial dementia. Binding was also demonstrated through immunoprecipitation of bPrP(T194A) and enzyme-linked immunosorbent assay. Immunoprecipitation of human embryonic kidney (HEK293T) cells stably expressing wild-type PrP^{C} or recombinant T194A PrP^{C} also confirmed binding of

SN6b to T194A PrP^C. Protein misfolding experiments conducted with SN6b and bPrP(T194A) did not promote formation of a protease resistant conformation alleviating some concerns that the interaction could accelerate disease onset. Collectively these findings may be of significance in the targeted application of disease specific immunotherapy to genetically at risk individuals for prion as well as other protein misfolding diseases. Although in humans the incidence of prion diseases with regard to genetic variants associated with PrP^C misfolding is low, these individuals represent an exclusive group, for human administration of the vaccine.

HD.15: Prion disease among Asians and Pacific Islanders in the United States, 2003–2009

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Introduction. Asians and Pacific Islanders (APIs) comprise approximately 5% of the United States population. The occurrence of Creutzfeldt-Jakob disease (CJD) and other prion diseases among APIs in the United States has not been widely investigated.

Materials and Methods. API prion disease decedents were identified from the United States national multiple cause-of-death data and the National Prion Disease Pathology Surveillance Center database for 2003–2009. Relevant portions of medical records and results from neuropathologic and genetic testing for API prion disease decedents were obtained and reviewed, as available.

Results. During 2003–2009, 60 API prion disease decedents were identified; 34 of these decedents had additional race information available, with 15 API decedents classified as Filipino (44%), 13 as Japanese (38%), 5 as Chinese (15%) and 1 as Hawaiian (3%). The average annual age-adjusted incidence was 0.7 per million population, significantly lower than that for whites ($p < 0.001$). Over half (55%) of the API deaths occurred in California or Hawaii, the states with the highest API populations. The median age at death was 66.5 y (range 40–87 y), similar to that for whites (68 y). Neuropathology reports and/or medical records were available for 24 of the decedents; for 20 cases with a reported disease onset date, the median duration of illness was 4.5 mo (range 1–66 mo). Twenty of 22 decedents (91%) had sporadic CJD, while the remaining 2 decedents (9%) had familial CJD. All 20 decedents with genetic testing results available were methionine homozygous at codon 129.

Conclusion. For 2003–2009, the reported prion disease incidence among APIs in the United States was significantly lower than that for whites. Underreporting of API race may contribute at least partly to this lower incidence, but genetic factors may influence prion disease susceptibility as well. Because the API race is heterogeneous, further study of prion diseases among specific API ethnic groups is warranted.

HD.16: vCJD associated with organ or tissue transplantation in the UK: A lookback study

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Introduction. Person-to-person transmission of vCJD has occurred via blood transfusion, and could also theoretically occur due to the transplantation of organs or tissues. The aim of this study was to investigate whether any UK cases of vCJD could have contracted vCJD by receiving an organ/tissue transplant, or have transmitted vCJD to others by this route.

Materials and Methods. A history of medical procedures for vCJD cases reported in the UK was reviewed to identify situations where the receipt or donation of organs and tissues might have occurred. If such a procedure was identified, a 'look-back' was then performed to determine if it was possible to identify the potential donors/recipients of organs/tissues to/from those people who went on to develop vCJD. This was in order that subsequent public health measures could be taken if necessary, in line with UK guidance.[1]

Results. Surgical histories were reviewed for 175 UK vCJD cases. Of these, a total of 137 patients had a history of surgical procedures, of whom 35 had undergone procedures where the donation/receipt of organs/tissues might have occurred. A further detailed investigation of the medical notes for these 35 patients identified one vCJD case as having undergone a liver transplantation (the same patient had also received red cells from 103 donors in transfusions prior to and following transplantation). The subsequent look-back was able to trace six other organ/tissue donations made by the same donor. No other situations where the donation/receipt of organs/tissues to/from people who went on to develop vCJD were identified. A public health notification exercise has been undertaken for the 103 blood donors who were considered "at risk" of vCJD. There was considered no need for further public health action in relation to the liver recipient or liver donor (deceased) or the recipients of other organs/tissues from the liver donor.

Conclusion. Organs and tissues are used in a wide variety of operations and it is important to continue to seek to identify individuals at risk of vCJD by this route so that appropriate public health measures can be implemented.

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HD.17: Enhanced surveillance of individuals identified as at increased risk of CJD in the UK thorugh iatrogenic exposure: An update

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Background. Surveillance of individuals at increased risk of CJD due to healthcare exposures is important to better understand the transmission and natural history of secondary infection and to monitor the effectiveness of public health measures to prevent on-going transmission of CJD. Individuals in at-risk groups have experienced a range of exposures, and probably have different risks of infection.

Materials and Methods. In the UK certain people identified with a risk of exposure to CJD above the background level for the UK population have been classified as "at increased risk of CJD for public health purposes." Data on individuals and groups of people identified in this way are managed and held by a number of different organisations depending on their risk exposure. Surgical and blood exposure details are held by the Health Protection Agency (HPA) and Health Protection Scotland (HPS) in collaboration with the TMER. Registers of hemophilia patients treated with UK sourced plasma products in the 1980s and 1990s and of recipients of human Growth Hormone before 1985 are held by the National hemophilia Database and the Institute of Child Health (ICH) respectively. We present a combined overview of these patient groups as an update on the enhanced surveillance of healthcare exposure to CJD in the UK.

Results. By the end of 2012, 6,143 people had been identified as at increased risk of CJD due to one or more healthcare exposures. Of those, 63% were recipients of UK sourced plasma products, 31% were treated with human Growth hormone, 2.5% had a surgical exposure, 1% received blood from a donor who later developed variant CJD and 2.8% through other healthcare exposures. 999 individuals are known to have died, 74 of whom developed and died from CJD (71 following treatment with human Growth hormone and three from variant CJD after receipt of labile blood, or components from a donor who later developed the disease). There have been no clinical cases in this cohort associated with other exposures to date. Most individuals are assessed as at risk because of historic exposures, the main ongoing risks being rare exposures following surgery on an individual later diagnosed with sporadic CJD or inherited prion disease and potentially from blood donation and surgery involving subclinical carriers of vCJD.

Conclusion. Continued long-term follow-up of individuals exposed to an increased risk of CJD remains important to monitor health outcomes at a national level.

HD.18: Creutzfeldt-Jakob disease reporting in Canada

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Background. To deal with risks of infectious transmission of Creutzfeldt-Jakob disease (CJD), in 1998 the Government of Canada launched a prospective national CJD surveillance system (CJDSS). In 2000, CJD became nationally notifiable in Canada, and since then all Canadian Provinces and Territories (P/Ts) have made CJD reportable. It has been recognized that the CJDSS registers more cases of CJD than are reported to P/T Ministries of Health (PTMH). Because the CJDSS may not legally share personal information with PTMH, in 2008 the CJDSS began to systematically discuss the issue of CJD reporting with referring health care professionals (HCP). The present study was undertaken to estimate any changes in P/T CJD reporting from 2008, and to identify possible areas for further improvement.

Materials and Methods. P/T CJD data were retrieved from the Public Health Agency of Canada's National Notifiable Disease System (NNDS) database, and compared with CJDSS data. CJDSS intake sheets were examined, to determine if the case had been reported to the PTMH at the time of notification.

Results. From 2005 to 2010, NNDS received complete data on CJD from 5 P/Ts. During the same period, 134 cases of CJD (probable or neuropathologically confirmed) were reported by the 5 P/Ts while 210 CJD deaths (probable or definite) were recorded in the CJDSS from the same 5 P/Ts. Between 2008 and 2010 there was an increase of ~48% in P/T CJD reports compared with the period 2005–2007. In contrast, the CJDSS registered only ~12% more CJD deaths between 2008 and 2010 compared with 2005–2007, supporting an interpretation of improved P/T reporting. Examination of intake sheets from 172 notifications that were made to the CJDSS from the same 5 P/Ts between 2008 and 2010 revealed that 30 were known to have been reported to PTMH at the time of referring (24 were CJD, 5 were non-CJD, and 1 was unclassifiable). 142 were not reported or had unknown reporting status. Reasons cited by HCPs for not reporting included (1) uncertainty of the CJD diagnosis; (2) uncertainty regarding responsibility for reporting; (3) lack of awareness that CJD is reportable; and (4) uncertainty regarding when or how to report.

Conclusion. The considerable increase of CJD reports in P/Ts since 2008 occurred concurrently with efforts of the CJDSS to engage HCPs on the issue of CJD reporting requirements. P/T CJD reports may include non-CJD cases. Inter-jurisdiction collaboration is underway to further improve CJD reporting.

HD.19: Doxycycline in Creutzfeldt-Jakob disease: A phase 2, randomized, double-blind, placebo-controlled trial

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Introduction. At present, there is no effective treatment to modify the course of Creutzfeldt-Jakob disease (CJD). Tetracyclines were found to bind amyloid fibrils of different composition, including PrP, and to reduce infectivity of prion-infected brains. Among tetracyclines, doxycycline (1) interacts with and revert the protease-resistance of PrP^{Sc} extracted from brain tissue of patients with all forms of CJD, (2) reduces the infectivity titer in prion-contaminated material and (3) prolongs survival of experimentally prion-infected animals. On this ground, a series of CJD patients in Italy and Germany received compassionate treatment with doxycycline at a daily oral dose of 100 mg from the time of diagnosis to death. The results of these open trials showed an increase in survival time in doxycycline-treated patients compared with historical series. This result prompted Italian and French CJD care systems to carry out a randomized, double-blind study of doxycycline vs. placebo with the primary objective to evaluate its effectiveness in increasing survival time in patients with CJD.

Materials and Methods. In both countries we included patients with a probable diagnosis of sporadic CJD and patients with genetic forms due to E200K mutation in France and E200K and V210I mutation in Italy, because of a clinical phenotype indistinguishable from that of sporadic CJD. Enrolled subjects were treated orally with 100 mg doxycycline per day or with identical placebo under double-blind conditions from the day of randomization to death. The primary efficacy variable was the survival time from the day of randomization. Patient and his family were informed on the protocol and a signed consent was obtained. The study was approved by the Ethics Committees of Carlo Besta Institute and the other involved Italian centers, and the Pitié-Salpêtrière Hospital.

Results. We randomized 121 individuals (55 in Italy and 66 in France). Of these patients, 62 were assigned to the treatment group and 59 to the placebo group. No differences in gender, age and PRNP codon 129 distribution were observed between French and Italian patients and between patients included in placebo or doxycycline group. The results on the effect of doxycycline on the primary efficacy variable will be presented and discussed.

Conclusion. This study is the first European double blind vs. placebo trial that enrolled a large number of patients with prion disease. It will pave the way to large-scale multi-national controlled trials in this rare pathologic condition that are mandatory for an accurate evaluation of forthcoming anti-prion molecules in humans.

HD.20: Voice recorded messages from significant others as an adjunct therapy to increase the level of consciousness of patients with reversible coma

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Keywords: reversible coma, level of consciousness, voice-recorded messages, auditory stimulation

Introduction. Hearing is the last sense that deteriorates when a person becomes unconscious. Meaningful auditory stimuli provide emotional arousal that brings about an increase in the level of consciousness.

Methodology. This study aims to determine the effects of the voice recorded messages from significant others as an adjunct therapy to increase the level of consciousness of patients with reversible coma. Using purposive sampling technique, 15 subjects were chosen with the following criteria: (1) in a reversible comatose state; (2) receptive to stimuli; (3) not in comatose state for more than 1 mo; (4) no left-sided brain affectation; (5) admitted in the stroke unit or medical ward of East Avenue Medical Center; (6) accompanied by relatives; (7) 18 y old and above; and (8) relatives able to sign the informed consent. The message lasts for 3–7 min and repeated 4 times daily for two weeks. The FOUR Score, GCS and Vital Signs of the patient will be assessed before and after the intervention.

Results and Discussion. Assessments were as follows: FOUR score scale ($p = 0.004$), GCS ($p = 0.000$), pulse rate ($p = 0.000$), respiratory rate ($p = 0.000$), temperature ($p = 0.655$), systolic blood pressure ($p = 0.196$), diastolic blood pressure ($p = 0.745$). P-value lesser than 0.05, showed a significant result.

Analysis and Conclusion. Voice recorded messages from significant others as an adjunct therapy showed improvement in the level of consciousness and vital signs of patients with reversible coma as evidenced by significant differences found in FOUR score, GCS, RR, PR and some behavioral responses observed such as jerking, groaning, crying, and having the patient's first movements of the day as verbalized by relatives.

HD.21: 2-aminothiazoles extend survival substantially in prion-infected mice

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Introduction. There is not a single approved drug that halts or even slows the progression of a neurodegenerative disease, including Creutzfeldt-Jakob disease (CJD). The only small molecule shown to extend survival of prion-infected mice is a biaryl hydrazone termed "Compound B"[1]. However, the hydrazone moiety results in toxic metabolites, making it a poor candidate for further drug development. We recently reported that compounds with the 2-aminothiazole scaffold showed good efficacy against prion-infected cells.[2] Medicinal chemistry optimization was used to determine structure-activity relationships and identify compounds that were orally absorbed and achieved high brain concentrations in animals.[3,4] Two compounds with good brain exposure and microsomal stability, IND24 and IND81, were tested in prion-infected mice.

Materials and Methods. Prion-inoculated mice were dosed continuously with IND24 or IND81 blended in a complete liquid diet. Treatment was started at various times after inoculation. Brains of treated mice were analyzed biochemically and neuropathologically to identify any changes in strain characteristics.

Results. Treatment with IND24 or IND81 of mice infected with the RML prion strain extended survival by ~80%, even when dosing was initiated as late as 60 d after inoculation. Brains of treated mice had a different PrP^{Sc} banding pattern and vacuolation profile, compared with those of vehicle-treated controls. Serial passage from the brains of treated mice demonstrated that continued treatment with IND24 was required for the maintenance of the new strain characteristics. Treatment with IND24 also led to an ~80% survival extension in mice infected with the ME7 prion strain when dosing was started immediately after inoculation. However, starting treatment at 60 d resulted in only a ~25% extension in survival, suggesting differing kinetics in propagation of the ME7 strain. We then tested IND24 in transgenic mice susceptible to sporadic (s) CJD prions. We did not observe extension in survival in mice infected with either the sCJD(MM1) or sCJD(VV2) strains, in multiple mouse models.

Conclusion. IND24 is the most efficacious compound reported to date for the treatment of prion infection in mice. However, the prion strain specificity observed holds important lessons for the future of drug development.

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HD.22: Amyotrophic lateral sclerosis (ALS) swine models: Production and preliminary characterization

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Keywords: amyotrophic lateral sclerosis (ALS), Cu/Zn superoxide-dismutase1 (SOD1), somatic cell nuclear transfer (SCNT), swine model

Introduction. Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease that occurs in two forms: sporadic and familial, the latter linked to mutations in the SOD1 gene. As employment of transgenic SOD1 rodent models in ALS research didn't result in an improvement of patient prognosis, another model, more closely related to human species, is strongly demanded by the scientific community. On this basis, our group produced, by Genetic Engineering and SCNT, transgenic blastocysts and swine carrying the hSOD1G93A mutation, which is the most frequently studied in rodents, since it reproduces patients phenotype progression.

Materials and Methods. SCNT blastocysts on fifth day of development were transplanted by midventral laparatomy to the synchronized sows uterus. A cesarean delivery was performed at the 114th day of gestation. In order to achieve a preliminary characterization of our swine model, tissue banking was performed on stillborn piglets and on animals that died soon after birth. Immunocytochemistry on ear biopsy fibroblasts and western blot on homogenized snap-shot frozen tissues (rabbit policlonal

antibody 07–403 Millipore, concentration 1:200 and 1:1000 respectively) were performed. To assess SOD1G93A deposition pattern Immunohistochemistry (rabbit policlonal antibody GTX 100659; 1:250) and Immunofluorescence (GTX 100659; 1:250 and NeuN MAB377; 1:1000) were employed on FFPE tissues. Genomic SOD1G93A swine DNA digested by SalI+BglII (10 U/μg DNA) was hybridized with SOD-DIG probes (20 ng/ml) to assess transgene integrations number by Southern Blot.

Results and Conclusions. The transfer of 638 embryos to eight recipient sows resulted in four pregnancies and in the birth of 16 vital and 12 stillborn piglets (mean blastocyst development to term efficacy, 8.78%). Five animals developed normally while the remaining piglets died due to events commonly reported in commercial herds. The transgenic protein expression was confirmed by both immunocytochemistry and western blot. Furthermore Southern blot revealed a transgene integration number ranging from 1 to about 6 copies. IHC demonstrated granular mutant protein aggregates in both perikarya and neurites of neurons (nucleus labeled with NeuN in immunofluorescence experiment) in brain (from area hypothalamica lateralis to the third ventricle) and in spinal cord neurites.

Despite these encouraging results, further molecular and pathological investigations are required since data have been obtained in stillborn or extremely young animals. A detailed phenotypical characterization, adapting to pig currently employed human diagnostic devices, is in progress on adult living swine.

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HD.23: Creutzfeldt-Jakob disease among American Indians and Alaska natives in the United States, 1983–2009

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Background/Introduction. Creutzfeldt-Jakob disease (CJD) occurrence among American Indians and Alaska Natives (AI/ANs) is of special interest, in part because of the high prevalence of hunting and venison consumption in this population. Such behaviors could place AI/ANs at increased risk of prion disease if chronic wasting disease (CWD) were found to transmit to humans.

Materials and Methods. Death records with CJD as any-listed cause of death for US residents identified from the national multiple cause-of-death data and other surveillance mechanisms for 1983 through 2009 were analyzed, and incidence was calculated by race. Available death certificates and medical records were collected and examined for AI/AN decedents.

Results. During 1983 through 2009, 15 decedents with CJD as a cause of death were reported as AI/AN race. The average annual age-adjusted CJD incidence for AI/ANs was 0.39 per 1,000,000 persons. The rate for whites (1.07) was higher compared with that for AI/ANs (RR = 2.9, 95% CI = 1.8–4.9) and blacks were similar (0.41; RR = 1.1, 95% CI = 0.7–1.9). The median age at death was 65 y (range 39–85 y), similar to those for whites and blacks (68 and 66 y, respectively); four (27%) AI/AN decedents were younger than 55 y of age. Most of the AI/AN decedents were males (60%). Decedents were reported from 13 states; none resided in the states with the longest known presence of CWD, Colorado, Wyoming, and Nebraska.

Conclusion. The reported CJD incidence for AI/ANs appears lower than that for whites and similar to that for blacks, although the CJD incidence for AI/ANs is likely underestimated due to racial misclassification of AI/ANs. Continued monitoring of CJD occurrence in this population is important as CWD spreads into new areas.

HD.24: Surveillance of Creutzfeldt-Jakob disease in Korea: Establishing the Korean National Creutzfeldt-Jakob disease Registry (KNCJDR)

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Keywords: prion diseases, Creutzfeldt-Jakob disease, surveillance, epidemiology

Background. The Korean National Creutzfeldt-Jakob disease surveillance has been operated by Korea Centers for Disease Control and Prevention (KCDC) since 2001. In accordance with the increasing public concern about CJD, updated and comprehensive surveillance was conducted under the name of "Korean National Creutzfeldt-Jakob Disease Registry (KNCJDR)". Our study presents its setup process and descriptive features of the Korean prion diseases registry.

Materials and Methods. The KNCJDR database has been established by merging and reviewing all the available CJD cases from national notifiable infectious diseases reports, previous surveillance database, mortality database by National Statistics Office, and medical record survey by neurologists network. The KNCJDR database consist of demographic findings, clinical signs (progressive dementia, myoclonus, visual signs, cerebellar signs, pyramidal signs, extrapyramidal signs, and akinetic mutism), test results (EEG, Brian MRI, and 14-3-3 protein detection in cerebrospinal fluid) and result of pathologic study.

Results. Total number of cases merged to the KNCJDR database was 578 cases since 1980. After excluding 155 cases with

incomplete information 7.1%, 56.1% and 10.0% was classified as definite, probable and possible CJD respectively. The majority of Korean CJD cases were sporadic (about 80.4%). The range of the incidence rate of nationwide CJD was 0.03–0.84 between 1980 and 2012. Mean age of onset was 64.1 ± 9.8 y (63.2 y for males and 65.2 y for females) old. Mean duration of illness was 10.3 ± 10.4 mo (range 1–79 mo). Annual incidence of CJD has continuously increased during this period, and onset of symptom tends to be acute or sub-acute.

Conclusion. Functioning KNCJDR is crucial for achieving effective prevention and management of CJD. Although, this registry is contained in currently available data, the authors expect that it is to be basic platform in both academic research and national control plan for CJD.

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HD.25: Estimation of the size of iatrogenic Creutzfeldt-Jakob disease associated with cadaveric dura mater transplantation in Korea

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Keywords: prion diseases, iatrogenic Creutzfeldt-Jakob disease, dura graft, modeling

Background. In Korea, a national Creutzfeldt-Jakob disease (CJD) surveillance system was operated by the Korea Centers for Disease Control and Prevention (KCDC) since 2001 and the Korean National Creutzfeldt-Jakob disease Registry (KNCJDR) was established by the KCDC in December 2012. Since 1987, Dura mater graft-associated iatrogenic Creutzfeldt-Jakob disease (iCJD) has been reported in many countries. The first case of iCJD in Korea was reported in 2011. This study was conducted to estimate the overall size of iCJD from dura graft in Korea using a mathematical model.

Materials and Methods. In order to estimate the overall size of the iCJD, we assumed the annual cases of dura mater graft between 1980 and 2000 based on the number of neurosurgery cases applying proportion of dura mater use estimated from Health Insurance Review Agency (HIRA) claim data set in Korea. We designed two mathematical projection models and there were two scenarios applied to estimate exposure period to risk factor in each model. The incubation period distribution was assumed to use the Weibull distribution with density function and log-logistic distribution with density function. The overall size of iCJD was estimated by Monte Carlo sensitivity analysis.

Results. Total number of neurosurgery during year 1980 and 2000 was estimated to be 424,451. Among them, 23.9% was estimated to be grafted by dura product. From 1980 until 2020, our model predicted the range of iCJD would be approximately 13.4–50.9. The estimated range of the cumulative number of iCJD to be caused by dura graft was approximately 12.4–44.8 until 2011.

Conclusion. We postulated that the occurrence of iCJD was expected to be in sharp decline after 2012 until 2020.

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HD.26: Nurr1 agonist acts as a neuroprotective agent by reducing reactive oxygen level

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Introduction. Parkinson disease (PD) as a second prevalence neurologic disorder has largely unknown pathology. The cardinal symptoms of tremor, dystonia and postural instability result from degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNpc). Oxidative stress has a key role in PD pathogenesis and they are generated by mitochondria and glial cells in this disease and have destructive effects on dopaminergic neurons by accelerating their degenerations.Nurr1 as neuclear receptor has critical role in stem cells differentiation into dopaminergic neurons and in this study we used its agonist to declare neuroprotective role of this receptor in Parkinson disease in vitro model.

Materials and Methods. The effects of Nurr1 agonist (6-mercaptopurine) in protecting against MPP+ toxicity was evaluated in PC12 cells by MTS assay and reactive oxygen species (ROS) level was measured by flow cytometry.

Results. 6-mercaptopurine could protect PC12 cells against MPP+ toxicity and reverse its toxicity effect after 24 h. Decreasing ROS level was detected in PC12 cells which were treated by Nurr1 agonist in the presence of the MPP+ by flow cytometry and confirmed this fact that Nurr1 can control ROS generation and oxidative stress in PC12 cells

HD.27: Non-CJD cases of a methionine-to-arginine substitution in codon 232 in the PRNP

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Background. A methionine-to-arginine substitution in codon 232 (M232R) in the PRNP is known to be one of the mutations which cause Creutzfeldt-Jakob disease (CJD). Most of the CJD cases have been reported in Japan in addition to Korea and China. However, not all the patients with M232R were diagnosed with CJD. Even normal individuals showed M232R, although almost

all of the normal subjects did not have M232R. We report 3 non-CJD patients and 1 normal individual with M232R in the PRNP.

Case Report. A 47-y-old man (case 1) presented with a 4-y history of slowly progressive memory impairment. He also complained mild way-finding problem. He noted difficulty with remembering details of recent event. His past medical history was unremarkable. No myoclonic jerks, weakness, rigidity or ataxia were observed. His baseline and 55 mo follow-up MMSE (Minimental state examination) score was both 27. Brain MRI, including diffusion-weighted imaging, revealed no abnormality. Brain 18-F-flurodeoxyglucose-PET (FDG-PET) showed mild hypometabolism in the bilateral parietal and anterior temporal lobes. He was diagnosed with mild cognitive impairment. His clinical course was stationary during the follow-up period of 55 mo.

A 52-y-old woman (case 2) presented with a 3-y history of progressive memory impairment. It was also hard for her to manage financial matters independently. Her past medical history was unremarkable. A neurologic examination revealed no focal signs. Her baseline and 12 mo follow-up MMSE score was 15 and 9, respectively. Brain MRI was normal. Brain FDG-PET revealed hypometabolism in the frontal, temporal and parietal lobes bilaterally. She was diagnosed with Alzheimer disease.

A 50-y-old woman (case 3) started to have chronic fatigue and memory impairment since 8 y before presentation. No myoclonic jerks, weakness, rigidity or ataxia were observed. Brain MRI revealed no abnormality. Brain FDG-PET showed mild hypometabolism in the bilateral parietal lobes. She was diagnosed with mild cognitive impairment. Her 65-y-old sister (case 4), who did not complain any symptom and did not show any focal sign on neurologic examination, also had M232R in the PRNP.

Discussion. Given that an amino acid at codon 232 is included in a glycosylphosphatidylinositol (GPI) anchor signal sequence which is cleaved from the mature cell surface prion protein, the pathogenicity of M232R is questionable. However, amino acid substitution in the GPI anchor signal sequence was reported to affect the GPI anchoring and the conversion efficiency of the prion protein. The meticulous follow-up of our cases might help us understand whether M232R in the PRNP is a pathogenic mutation for CJD or a rare polymorphism.

HD.28: Deconvolution of molecular targets for small molecule anti-prion compounds using proteomic and microarray techniques

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Introduction. Many classes of anti-prion compounds have been reported in the literature but so far no compounds have been found that are effective in vivo. This may be because of low potency, lack of discernible structure-activity relationship or issues with toxicity and undesirable pharmacokinetic properties. We have published work describing the activity of a family of indole derivatives as anti-prion agents using a SMB cell line (non-neuronal mouse brain cells persistently infected with Scrapie which continuously produce PrP^{Sc}).[1-3] This study reports the deconvolution of cellular targets for these indole derivatives using a systems approach, i.e., proteomics and microarray techniques, and the subsequent validation of possible targets.

Materials and Methods. *Proteomic and microarray assays.* SMB cells were treated with either DMSO (negative control), quinacrine (positive control) or one of two of the lead indole compounds. Biological replicates were used and compound concentration and time of treatment were determined prior to sample preparation. Changes in the protein expression profile after treatment was determined using an iTRAQ based proteomic workflow. For the microarray work, RNA was extracted and applied to the appropriate Gene Chip array. The fluorescent signal was developed and data acquired using the Expression console package. *Validation*. Known inhibitors of the proteasomal pathway (MG132 and PMSF) were used in a competitive assay with the lead indole to assess the effect of inhibition on activity in the cell model. Western blot analysis was performed on targets identified by network analysis to confirm changes in expression.

Results. A cellular level biological network was built and possible drug targets for the anti-prion compounds were identified for further validation. The proteasomal pathway was identified from this analysis as being affected by treatment, consistent with other studies showing that proteasomal dysfunction increases accumulation of PrP^{Sc}.[4] Competition assays show that MG132 reduces the potency of indole compounds via a possible antagonist mechanism.

Conclusion. A cellular level biological network was built and possible drug targets for the anti-prion compounds were identified for further validation. The proteosomal pathway is currently being investigated and further validation is required to confirm the findings.

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HD.29: Doxycyclin is prolonging life in patients with CJD

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Keywords: prion, Creutzfeldt-Jakob disease, treatment

Introduction. Prion diseases are fatal spongiform encephalopathies. In several animal studies substances have been tested for treating prion diseases. Among them the most promising substance was tetracycline (Doxycyclin) because of its anti-prion effect, the good tolerance, and efficacy. In our study we show that the progression of the disease can be slowed down and survival increased following treatment with Doxycyclin.

Materials and Methods. All patients with probable or possible CJD that were treated with Doxycyclin 100 mg daily dose for at least 30 d were included: 65 patients (men, n = 41; woman, n = 24), mean age: 63 y. The patients were selected from the National Reference Center of TSE Surveillance and split into 3 groups. Group 1 was recruited in the time period from 06/2006 to 08/2008 (n = 28), group 2 in the time period from 09/2008 to 12/2012 (n = 25) and group 3 is a double- blinded randomized study with n = 13 patients recruited in 2010/2011. Patients were additionally characterized by codon 129 polymorphism and MRI signal intensity.

Results. We demonstrated a significant effect of Doxycyclin in relation to disease progress (slower) and survival (longer) in all groups tested here compared with controls.

Conclusions. The effect of Doxycyclin on disease progression was modified by age and codon 129 genotype. Although control trials are extremely difficult to perform in such rare conditions and although they are challenging with respect to for logistic, statistical and financial reasons, our experience shows that they are feasible. Unless new drugs become available to treat these devastating human disorder, in early disease stages administration of Doxycyclin in patients is recommended because of extremely good tolerability and apparent effect on disease progression.

HD.30: Conformation dependent immunoassay can distinguish different PrP^{Sc} types within individual brains from patients with variably protease-sensitive prionopathy

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Background and Introduction. Variably protease-sensitive prionopathy (VPSPr) is a novel human prion disease that was first reported in the USA.[1] Further cases of VPSPr have been reported in the USA,[2] UK,[3] The Netherlands[4] and Spain.[5] A defining feature of VPSPr is the presence of the abnormal form of the prion protein (PrP^{Sc}) that is less resistant to proteases, resulting in a faint ladder of bands including one at 8kDa on western blots following proteinase K (PK) digestion. Conformation dependent immunoassay (CDI) is based on the use of a detection antibody that binds to an epitope that is concealed in the native form of PrP^{Sc}, but exposed after denaturation with guanidine HCl. CDI detects both protease-sensitive and -resistant PrP^{Sc} but its use in the analysis of VPSPr cases has not yet been reported.

Materials and Methods. Frontal cerebral and cerebellar cortex tissue from four UK cases and one Dutch case of VPSPr were analyzed by CDI. Four of these cases were VV at *PRNP*-codon 129 and one was MV. Homogenates were analyzed following digestion with 0, 2.5 or 50 µg/ml proteinase K (PK). CDI uses a capture antibody that binds the C-terminus of PrP and is therefore unable to bind to the 8 kDa mentioned above.

Results. With no protease treatment, both PrP^{C} and PrP^{Sc} were detectable in all samples from all VPSPr cases at levels comparable to sporadic Creutzfeldt-Jakob disease. A significant proportion of PrP^{Sc} was eliminated after treatment low PK (2.5 µg/ml). In the frontal cortex, all PrP^{Sc} detectable by CDI was eliminated by 50 µg/ml PK, the standard concentration used for WB analysis. In contrast, a small proportion of PrP^{Sc} detected in the cerebellum of two of the cases was found to be resistant to this treatment. Interestingly, WB analysis showed different banding patterns in the cerebrum and cerebellum, with a predominance of three higher molecular mass (~20–30 kDa) bands in the latter.

Conclusion. Protease-sensitive PrP^{Sc} is, as expected, readily detectable in cases of VPSPr. However in the cerebrum and cerebellum in two cases PrP^{Sc} appears to differ in its ability to be detected by CDI after treatment with proteases. We conclude that CDI using a range of PK concentrations can discriminate between two distinct forms of PrP^{Sc} within individual cases of VPSPr.

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HD.31: p62 immunoexpression in human prion diseases with amyloid plaques

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Amyloid plaques are a hallmark of transmissible and non-transmissible brain amyloidoses, and in human transmissible spongiform encephalopathies or prion diseases occur in sporadic CJD (sCJD), kuru (both of which may contain kuru plaques), Gerstmann-Sträussler-Scheinker disease (GSS) (multicentric plaques) and variant CJD (vCJD) (florid plaques). P62/sequestosome is a ubiquitin-binding scaffold protein that co-localizes with many aggregated proteins in neurodegenerative diseases. It also links the ubiquitinated proteins to autophagic machinery and is aggregated when autophagy is inhibited. To continue our previuos work on autophagy in prion diseases[1,2] we decided to analyze the presence of p62 aggregates in prion diseases. Immunohistochemistry and immunofluorescence for confocal laser microscopy was performed in one case of vCJD, two sCJD cases, two GSS cases and one case of kuru. In each case, cerebellum and frontal cortex were studied. All the blocks used for immunochistochemistry were obtained from autopsy, routinely fixed in 4% formaldehyde and embedded in paraffin. Immunohistochemistry showed p62-positive structures around the plaques in all analyzed cases of prion diseases. Some p62-positive structures were also dispersed in the cerebtal and cerebellar cortices. We also observed some intraneuronal p62 postive structures. Confocal laser microscopy showed co-localization of p62 and neurofilaments (SMI31, SMI32) and to a lesser degree PrP^{Sc} and p62, βAPP with p62 and ubiquitin and p62. There was no col-localization of hiperphosphorylated tau (AT8) and p62, although, as we already published,[3] tau immunoexpression was present around plaques in all prion diseases with these structures. These findings suggest that immunoexpression of p62 is one of the markers of neuritic dystrophy but it's independent of tau expression which was also reported to be accumulated in dystrophic neurites. We also show intraneuronal p62positive inclusions in prion diseases character of which should be elucidated in further studies.

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HD.32: Presence of subclinical abnormal prion protein in all PRNP genotypes: Results of a second United Kingdom survey of archived appendix specimens

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Background. Widespread dietary exposure of the UK population to bovine spongiform encephalopathy (BSE) prions in the late 1980s and early 1990s led to the emergence of variant Creutzfeldt-Jakob Disease (vCJD). A distinct feature of this form of prion disease is the extensive involvement of the lymphoreticular system, which does not occur after transmission through contaminated human growth hormone or in Kuru. Accumulation of vCJD prions in spleen, tonsils or lymphoid tissue of the gastrointestinal tract, precedes invasion of the central nervous system. Thus, tonsil biopsies proved a specific and sensitive preclinical test for the detection of vCJD prions in patients, and the prevalence of vCJD carrier status in the population was estimated through screening archival appendectomy and tonsillectomy specimens. In view of differences between the findings in previous studies, a further large scale survey of appendix tissue was undertaken.

Materials and Methods. Paraffin blocks of appendix samples were collected from Pathology departments throughout the UK, were unlinked and anonymised, and then tested using immunohistochemistry for the presence of abnormal prion protein.

Results. In 32441 formalin fixed paraffin embedded (FFPE) appendix samples, we found 16 positive specimens, indicating a prevalence of 493 per million population, with a 95% confidence interval of 282–801 per million. The prevalence in those born in 1941–60 (733 per million; CI: 269 to 1596 per million) was not significantly different from those born between 1961 and 1985 (412 per million; CI: 198 to 758 per million) and it was similar in both genders and across the three broad geographic areas sampled. Genetic testing for the PRNP codon 129 genotype revealed a high proportion of 129VV compared with the frequency in the normal population, and in stark contrast to confirmed clinical cases of vCJD who have all carried the PRNP 129MM genotype. This study corroborates previous studies and suggests a remarkably high prevalence of subclinical vCJD prion infection in the population compared with the 176 vCJD cases to date. These findings have important implications for the surveillance and control of blood and blood products and for the handling of surgical instruments.

This project was funded by the Policy Research Programme of the Department of Health, England. Further studies are being commissioned in order to test the hypothesis that the prevalence is linked to the epidemic of BSE in cattle. Appendix samples archived prior to the BSE outbreak and from those born in 1996 or later will be tested.

HD.33: PrP loss of function in zebrafish: Relevance to Alzheimer disease

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Background and Introduction. Our immediate goal is to understand the normal biological role of the prion protein, with the longer term view that this will impact the development of therapeutics for prion and Alzheimer disease. Here we focus on loss-of-function approaches in zebrafish, because any phenotypes that are revealed will suggest ancient/important functions of PrP. Zebrafish possesses two homologs of *PRNP* (*prp1* and *prp2*). These can be replaced by mammalian *PRNP*, strongly supporting the conclusion that zebrafish homologs share conserved functions with mammalian PrP. Our recent demonstration of a genetic interdependence between homologs of *PRNP* and *APP* in zebrafish further supports a broad ancient role for PrP in the biology of Alzheimer-related proteins.

Materials and Methods. We have recently engineered zebrafish with a knockout of the gene *prp2*. Here we explore for phenotypes in this fish using behavioral assays, characterizing gene expression, and developing novel imaging approaches to post-larval brain development.

Results. In sharp contradistinction to phenotypes observed when *prp1* is disrupted by morpholino gene knockdown (including dramatic effects on early embryonic and later CNS development), it was challenging to identify phenotypes in our *prp2*$^{-/-}$ mutant zebrafish. We will review the possible explanations for these disparate outcomes that may be related to the timing of gene expression and/or gene sub-functionalization. Our new methods allow unprecedented imaging of post-larval brain development but do not reveal overt phenotypes in *prp2*$^{-/-}$ mutant zebrafish. Behavioral assays including application of a convulsant suggest that *prp2*$^{-/-}$ mutant zebrafish are susceptible to seizures.

Conclusion. The data argue for an ancient/important role for PrP in seizure susceptibility and neuronal hyper-excitability. Our zebrafish engineered to have mutations in *prp2* have phenotypes akin to *PRNP* knockout mice, but divergent from those observed following knockdown of zebrafish *prp1*. These results provide a promising path forward to understand the role of PrP in Alzheimer disease etiology, especially regarding excitotoxicity

HD.34: Differential proteome analysis of cytoskeleton associated proteins in the liver of PrP knockout mice

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Keywords: PrP^{C}, liver, differential regulation, cytoskeleton proteins, 2D gel electrophoresis, LC-MS/MS

Among peripheral organs, liver has recently been highlighted to be involved in the neurodegenerative diseases.[1,2] Recent reports also demonstrated the accumulation of misfolded form of PrP^{C} (causative agent of prion diseases) in the liver of sheep at both clinical and preclinical stages of the disease. In this study, we aimed to investigate tissue specific PrP^{C} dependent differential proteome regulation. We used gender and age matched PrP knock out and wild type mice. Samples were separated with 2D gel electrophoresis followed by differential proteome analysis with Decodon software. Proteins differentially regulated in the liver of PrP Knockout mice were identified by MS/MS and were subsequently verified by western blot. In conclusion, the present study demonstrates for the first time gender/age dependent significant regulation of proteins involved in cytoskeleton homeostasis. This study could provide important information regarding various cellular events mediated through cytoskeleton in prion diseases.

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HD.35: Characterizing the role of Hsp31 in modulating Sup35 prion aggregation

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Prions are self-propagating proteins that form amyloid aggregation and have been implicated in many neurodegenerative diseases in mammals and cause heritable traits in Saccharomyces cerevisiae. Yeast provides a useful model to understand the underlying mechanism of prion aggregation. In yeast, [PSI+] prion is the aggregated form of translation termination factor Sup35. Molecular chaperones such as heat shock proteins acts as a cellular defense system that protects the cell from diverse environmental conditions by different mechanisms including the modulation of protein conformation. Hsp31 is a member of small heat shock proteins that has structural similarity with human DJ1 and E. Coli Hsp31 proteins. Recently, it has been shown that Hsp31 has a role to protect the cells against oxidative stress. Our lab has shown that Hsp31 protect cells against α synuclein toxicity by preventing formation of amyloid aggregates in yeast. Hsp31 possesses a chaperone like response as determined by in vitro fibrillization against α-synuclein as well as in a citrate synthase aggregation assay. In this study, we established the role of Hsp31 in preventing the Sup35 aggregation using fluorescence microscopy and flow cytometry. However, overexpression of Hsp31 did not prevent the induction of prion in yeast. In conclusion, we propose a model that Hsp31 could inhibit Sup35 aggregation with no effect on the intermediate "propagon" stage that

is postulated to induce prion formation. The investigation of this model has implications in understand prion formation and also intermediate oligomer forms of α-synuclein fibril formation.

HD.36: Opposite effect of PrPN1 and shed PrP on the formation of cell-derived SDS-resistant amyloid β species

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Alzheimer disease is the leading cause of dementia worldwide and no efficient cure is available. According to recent evidence, secreted forms of the prion protein, i.e., shed PrP and the N-terminal fragment PrPN1 are able to neutralize the toxicity associated with amyloid β (Aβ) oligomers. In vitro assays with synthetic Aβ and recombinant PrP and PrPN1 indicate that both molecules block the polymerization of Aβ into amyloid fibrils. However, this anti-amyloid activity of PrP and PrPN1 has not been shown with cell-derived Aβ species.

Here, we used conditioned media from CHO-7PA2 cells that overexpress a familial mutant of APP ($APP_{Indiana}$) as a source of cell-derived Aβ species. We show by size exclusion chromatography followed by SDS-PAGE and western blot that secreted Aβ monomeric species completely polymerize into soluble high molecular weight SDS-resistant forms within 4 h. This simple assay with physiologically relevant Aβ species was used to test the anti-polymerization activity of PrP in subsequent experiments. In the presence of conditioned media from cells expressing PrP, Aβ monomeric species remained stable and did not polymerize. Since PrP expressing cells secrete both PrP and PrPN1, we tested the effect of recombinant PrPN1 or shed PrP on the polymerization of cell-derived Aβ monomeric species. Unexpectedly, we found that recombinant PrPN1 potently inhibit the formation of SDS-resistant Aβ species, while recombinant shed PrP favored the assembly of such species. These data demonstrate opposite effects of PrPN1 and shed PrP on the polymerization of cell-derived Aβ.

Our findings with cell produced Aβ confirm that PrPN1 is a potent inhibitor of Aβ polymerization into high molecular weight and SDS-resistant species. Since PrPN1 results from the α-cleavage of full length PrP by an unknown protease, the identification of this protease may help developing a new class of therapeutic agents for Alzheimer disease.

HD.37: Prion therapeutic efficacy is limited by the emergence of a drug-resistant strain

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Many attempts have been made to identify compounds that are effective against the disease-causing prion protein (PrP^{Sc}). While many compounds clear PrP^{Sc} in cell models, few have produced meaningful extensions in the lifespan of mice. Compounds with the 2-aminothiazole scaffold have been shown to effectively reduce PrP^{Sc} in cells,[1] and two of these compounds, IND24 and IND81, have been tested in prion-infected mice. Treatment of RML-infected mice with IND24 or IND81 extended survival by as much as ~80%. However, despite the dramatic extensions in lifespan, treated animals nonetheless died of prion disease. We characterized the PrP^{Sc} that persisted in the brains of mice treated with IND24 or IND81, by biochemical and neuropathological analyses as well as through serial passage into additional animals and infection in N2a and CAD5 cell lines. We found that PrP^{Sc} from treated animals had significantly altered glycoform ratios, a distinct pattern of PrP^{Sc} deposition and vacuolation, and an altered ability to infect cultured cells. Whereas both N2a and CAD5 cells were susceptible to RML infection, only CAD5 was able to propagate the PrP^{Sc} from drug-treated animals. Moreover, these prions developed resistance to IND24; that is, subsequent treatment with IND24 no longer cleared cells of PrP^{Sc}. These traits were maintained upon second passage in the presence of IND24, but returned to RML-like characteristics in the absence of IND24. These results demonstrate the emergence of a drug-resistant prion variant upon treatment with a highly efficacious antiprion compound, and have implications for future drug discovery efforts.

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HD.38: MicroRNA regulation of NMDA receptors during prion influenced neurodegeneration

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Background. The discovery of microRNAs (miRs) has revolutionized our understanding of gene regulation. MiRs bind to target sequences in mRNAs, preventing translation; a single miR may bind up to 200 functionally diverse gene targets. Therefore, the number of regulatory circuits effected by miRs is huge, perhaps as extensive as by transcription factors. An emerging role for miRs has been identified in various neurodegenerative diseases including prion disease.

We have previously reported differential miRNA regulation in the whole brain of mice during RML Scrapie infection. Two highly conserved miRs, miR-128 and miR-342-3p, were upregulated in the whole brain of animals infected with mouse-adapted scrapie, bovine spongiform encephalopathy (macaques) and Creutzfeld-Jakob disease (humans). Using bioinformatic prediction we found that these 2 miRs target GRIN2B and GRIN2D which are components of the N-Methyl-D-Aspartic acid receptor (NMDAR) and are important in neurotransmission and synaptic plasticity. Significantly, the normal form of prion protein, PrP^{C}, has been shown to attenuate excitotoxicity by inhibiting NMDARs. Dysregulation of excitoxicity related gene expression has been observed in the CA1 region of the hippocampus during early stages of mouse scrapie infection in our laboratory.

Hypothesis. MiRs found upregulated in prion disease, namely miR-128 and miR-342–3p, regulate NMDARs in homeostatic conditions and become disrupted in prion influenced neurodegeneration due to NMDAR activation.

Materials and Methods. Primary mouse Hippocampal (PMH) or cortical neurons (PMC) were treated with glutamate to elicit neuroprotective or neurotoxic pathways and miRNA expression profiling was performed using qPCR and microarray analysis. The GRIN2B and GRIN2D 3′ untranslated regions were tested for miR-128 and miR-342–3p binding by luciferase reporter assay in HeLa cells. MiR-128 overexpression or knockdown in PMH and PMC by pre-miRs and anti-miRs was performed and NMDAR transcripts and protein detected. Survival of primary mouse neuronal cells during excitotoxic challenge will be determined after overexpression or knockdown of candidate miRNAs. Correlations of in situ hybridization of miR-128 with immunohistochemistry of GRIN2B or GRIN2D were performed in RML Scrapie infected mice at preclinical and clinical timepoints.

Results. MiR-128, but not miR-342–3p, targets GRIN2B and GRIN2D by Luciferase Assays. PMC and PMH neurons also demonstrate highly significant decreases ($p > 0.0001$) in GRIN2D but not GRIN2B mRNA transcripts in miR-128 overexpression experiments. Knockdown experiments show slight but significant changes in GRIN2A, 2B and 2D mRNA transcripts.

Conclusion. Our results suggest a relationship between miR-128 and NMDAR subunit regulation during prion disease. Further work will reveal insights into this relationship.

HD.39: Transmission studies of an atypical Italian sCJD case show propagation of multiple prion strains

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In 2007, we described a novel PrP^{TSE} glycotype (PrP^{TSE} type U) in a patient, methionine/valine (M/V) at codon 129, with sporadic Creutzfeldt-Jakob disease. PrP^{TSE} was characterized by marked under-representation of the fully glycosylated isoform and PK cleavage sites at G67, G72 and G90. Nervous tissue immunostaining disclosed a granular, intraneuronal and intra-axonal patterns of PrP deposition.[1] Biochemical studies confirmed that conformational properties of PrP^{TSE} type U were distinct from PrP^{TSE} type 1 and type 2, including differences in PrP^{TSE} aggregate sedimentations as well as resistance to protease digestion under increasing guanidine hydrochloride (GdnHCl) concentrations. In addition, under denaturing conditions, i.e., in GdnHCl exposure followed by protease digestion, PrP^{TSE} type U unglycosylated band separated into two PrP^{TSE} conformers migrating at 23 and 20 kDa with distinct resistance to protease digestion. Transmission studies to voles were successful showing incubation periods and lesion profiles distinct from those observed in voles challenged with typical sCJD subtypes and PrP^{TSE}. In 10 out of 15 infected voles, Immunocytochemistry showed an intraneuronal pattern of PrP deposition not previously observed in voles infected with typical sCJD subtypes, but similar to that of the donor. In contrast, transmission studies into gene-targeted transgenic mice (Tg) carrying human PrP gene (*PRNP*) with all the possible combination at codon 129 were successful only in TgHuVV mice in 4 out of 18 mice. These mice showed a PrP^{TSE} with the same glycotype of PrP^{TSE} Type U. Our results indicate that PrP^{TSE} Type U is composed by two conformers propagating with distinct strain properties and shows different transmission properties form those observed in different sCJD subtypes challenged in voles. Previous experimental transmission studies of scrapie-derived strains to Tg mice, carrying PrP mutations at N-linked glycosylation sites, generated an incompletely glycosylated PrP^{TSE}. Here, in the presence of a PrP^{TSE} defective in glycosylation, we recovered a fully glycosylated PrP^{TSE} in voles and a

defective glycosylated PrP^{TSE} in TgHu mice indicating that prion replication might depend on either host or on the template.

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HD.40: Dynamic transcriptional regulatory networks in prion diseases

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Complex diseases such as neurodegenerative disease involve sequential, coordinated activation of pathophysiological processes during disease progression. However, the transcriptional regulators and interactions among them that may define the coordinated activation are still elusive in many complex diseases. Here, we present dynamic transcriptional regulatory networks (TRNs) formed by regulatory network motifs that are defined as a sets of molecules and their interactions, acting as basic regulatory units that can account for the sequential, coordinated induction of genes in prion infection. Using time-course gene expression profiles from three mouse strains infected with the RML prion strain, we selected 883 genes that showed early or late changes in expression. Using transcription factor (TF) interaction data for these differentially expressed genes (DEGs), we identified 59 TFs that could account for their transcriptional regulation and thus their associated cellular processes in prion diseases. By using transcriptional relationships among these 59 TFs and the early and late DEGs, we were able to identify 13 types of regulatory motifs that could account for coordinated changes in the neworks. Among these motifs, coherent and incoherent feed-forward loops were over-represented. These over-represented loops provide potential mechanisms underlying differential temporal activation of genes during prion disease progression, such as the late induction of genes reflecting neurodegeneration in prion diseases. Therefore, our systems approach provides a basis for developing transcriptional regulatory models for the coordinated activation of disease-perturbed cellular processes.

HD.41: Species barrier between cervids and humans demonstrated by in vitro seeding

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Prion diseases are transmissible spongiform encephalopathies in humans and animals, including scrapie in sheep, bovine spongiform encephalopathy (BSE) in cattle, chronic wasting disease (CWD) in deer and Creutzfeldt-Jakob disease (CJD) in humans. The hallmark of prion diseases is the conversion of the host-encoded prion protein (PrP^{C}) to its pathological isoform PrP^{Sc}, which is accompanied by PrP fibrillation. The conversion is induced by PrP^{Sc} invading the organism during the infection.

Transmission is not restricted within one species, but can also occur between different species. In some cases a species barrier can be observed which results in limited or even unsuccessful transmission. The mechanism behind interspecies transmissibility or species barriers is not completely understood.

To analyze the species barrier phenomenon, an in vitro fibrillation assay was established, in which recombinant PrP (recPrP) can be specifically seeded by PrPSc.1,2 The interspecies transmission to humans was analyzed by combining seeds from the species cattle, sheep and deer (BSE, scrapie, CWD) with human recPrP (huPrP). Intraspecies seeding served as a control (Luers L, et al. Submitted).

The results of the in vitro fibrillation assay are in clear agreement with epidemiological data and bioassays investigating the transmission of prion diseases between humans, cattle, sheep and deer. In contrast to CJD and BSE seeds, which clearly show a seeding activity in huPrP we can demonstrate a species barrier for seeds from scrapie and CWD. Therefore our data strongly support the hypothesis that CWD is not transmissible to humans.

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HD.42: Micrornas deregulated early in prion disease may contribute to a neuroprotective process

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Background. Currently, the molecular process governing synaptic dysfunction and neuronal loss during prion-induced neurodegeneration remains largely unknown. Investigating molecular

changes that occur early in disease, when the infectious prion protein is beginning to accumulate, may hold promise in identifying these disease-related pathways.

We performed extensive high-throughput transcriptomic and miRNomic temporal screens on a neuronal-rich brain region (CA1 hippocampus). From this screen, we identified the presence of a neuroprotective response that occurred during early, preclinical disease which diminished as disease progressed.[1] This protective mechanism was evident not only at the transciptomic level but also at the microRNA (miRNA) level. In fact, out of the 7 miRNAs that were upregulated during early prion disease, 3 miRNAs have known neuroprotective functions. We hypothesize that the remaining 4 deregulated miRNAs also contribute to the neuroprotective process.

Materials and Methods. Real-time PCR and in situ hybridization for the 4 candidate miRNAs (miR-16, miR-26a, miR-140 and miR-146a) was used to further validate the expression levels of these miRNAs in prion infected mice, specifically in the CA1 hippocampal brain region. MiRNA target prediction programs were employed to discern neuronal specific genes that are regulated by these miRNAs. These target lists were further refined by using mRNA microarray data. Currently, we are performing Luciferase reporter assays to validate the top candidate targets. To identify potential effects of these miRNAs on neuronal morphology, overexpression and knock-down experiments for each miRNA will be performed in primary mouse hippocampal cultures.

Results. We confirmed the upregulation of 4 miRNAs in early prion-induced neurodegeneration using real-time PCR and in situ hybridization techniques. Based on bioinformatic predictions, targets of these 4 candidate miRNAs were strongly associated with neuronal function by affecting dendrite formation. Therefore, we expect to observe a change in neuronal morphology after manipulating the concentration of these candidate miRNAs in primary mouse neuronal cultures. Currently, we have successfully optimized our primary mouse neuronal cultures in the laboratory and we are in the process of testing the effects of these miRNAs on dendrite complexity (Sholl analysis) and spine morphology (shape, volume, number).

Conclusion. We have shown that numerous miRNAs were involved in a neuroprotective program long before clinical symptoms were apparent in an animal model of prion disease. We believe that miR-16, miR-26a, miR-140 and miR-146a also exhibit neuroprotective properties and their contribution to this protective process remain the focus of further study.

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HD.43: PrP^{C} modulates A-type K^{+} currents through dipeptidyl aminopeptidase-like protein 6

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The cellular prion protein (PrP^{C}) is broadly expressed in the adult central nervous system and associated with a variety of processes including neuronal excitability. Dipeptidyl aminopeptidase-like protein 6 (DPP6) was first identified as a PrP^{C} interactor using in vivo formaldehyde crosslinking of wild type (wt) mouse brain. This interaction was confirmed in three heterologous cell lines and because DPP6 directs the functional assembly of voltage gated K+ channels, we assessed PrP^{C}'s impact upon Kv4.2-based cell-surface macromolecular complexes. Wt PrP^{C} modulates Kv4.2 channel properties causing an increase in peak amplitude, rightward shift of the voltage-dependent steady-state inactivation curve, slower inactivation and a faster recovery from steady-state inactivation. This effect upon A-type voltage gated K^{+} channels occurs only in the presence of DPP6 and may influence the heightened vulnerability to drug-induced seizures observed in Prnp$^{0/0}$ mice as well as implicate the regulation of Kv4.2 channels by PrP^{C} as a mechanism that could potentially contribute to the PrP^{C} dependent effects of oligomeric Aβ assemblies. Contrasting with wt PrP^{C}, a Gerstmann-Sträussler-Scheinker disease version of PrP with eight extra octarepeats exhibited loss-of-function both for complex formation and for modulation of Kv4.2 channels.

HD.44: The PrP-LZT relationship: Toward a physiological role of PrP

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We recently documented the co-purification of members of the LIV-1 subfamily of ZIP zinc transporters (LZTs) with the cellular prion protein and, subsequently, established that the prion gene family descended from an ancestral LZT gene. In subsequent biochemical studies we established that starving cells of manganese or zinc, but not copper, causes shedding of the disordered N-terminal domains of PrP^{C} and LZTs (of note, an

analogous endoproteolysis of the PrP-like ectodomain of LZTs can also be observed in prion-infected mice). Taken together with recent observations by others of zinc-dependent PrP dimerization a model emerges that sees PrP act as a sensor or modulator of the cellular cation homeostasis. Unclear at this time are not only the immediate consequences of PrP expression to this aspect of cellular biology but also the broader role it may play in cell fate decisions. To begin to address this question we have established biochemical assays that can measure the cellular uptake of divalent cations and correlate this information with the quantity of cell surface expressed PrP and ZIPs in naïve or prion-infected cells. By applying these assays to a range of specific ZIP and PrP expression constructs, we can determine the contribution of individual protein domains within these proteins to the cellular metal uptake. When combining this information with the monitoring of cell fate reporters we can begin to delineate the downstream cellular pathways that are influenced by this cation biology.

HD.45: The cellular prion protein enhances lactate dehydrogenase expression under hypoxic/ischemic conditions

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Background. The exact physiological function of the cellular prion protein (PrP^C) is not completely understood. Its neuroprotective functions have already been described. The occurrence of PrP^C appears to be advantageous for neuronal survival upon hypoxic/ischemic insult. In a model of cerebral ischemia prion-knockout mice exhibit significantly greater lesions as compared with wild-type mice. Revealing the molecular mechanisms underlying prion-mediated neuroprotection following ischemic injury is of significant interest.

Materials and Methods. Primary cortical neurons derived from wild type and PrP-knockout (Prnp0/0) mice. Both Prnp0/0 and WT mice were derived from 129/Sv and C57BL/6 genetic backgrounds. Prnp0/0 mice were generated as described earlier.[1]

In addition, transiently PrP^C transfected HEK293 cells were used to analyze the effect of PrP^C overexpression. Both cell types were subjected to hypoxia-re-oxygenation treatment. Afterwards, the expression of lactate dehydrogenase (LDH) was analyzed by western blotting.

Results. The main objective of the present study was to investigate potential differences in LDH protein expression levels employing wild-type and cellular prion protein knockout models under normoxic/hypoxic conditions. Under normoxic conditions, we obtained no significant differences in lactate dehydrogenase expression. Interestingly, a significantly increased LDH level was found after 60 and 90 min of hypoxia in wild-type compared with prion-knockout primary cortical neurons. WT neurons exhibited less hypoxia-induced damage as compared with knockouts.

LDH expression level was additionally analyzed following hypoxia in HEK293 cells overexpressing PrP^C in comparison to control cells expressing endogenous cellular prion protein levels. Likewise, HEK293 cells exhibiting an upregulation of PrP^C showed a higher LDH expression after 90 min hypoxia as compared with control cells.

Conclusion. Alltogether, our data showed an important role of LDH and possibly its product lactate in PrP^C-mediated neuroprotection under hypoxic conditions.

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HD.46: Large-scale shRNA library screen using a novel cell assay system identified Ube2cbp as a potential mediator of prion-associated neurodgeneration

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The invariably fatal neurotoxic nature of the prion agent is associated with an alternative secondary structure of the host encoded prion protein. The molecular mechanism by which the disease-associated prion protein exerts its neurotoxic effect remains elusive. To date, the only protein known to be essential for prion disease susceptibility is the prion protein. One major hurdle in the identification of genes whose products play a role in the underlying molecular dysfunction associated with the misfolded prion protein, is the lack of a sufficiently neurotoxic cell culture model to permit the identification of other disease-associated gene products. Here, we describe the development and use of the first cell culture model to meet these criteria.

Using the OpenBiosystems shRNAmir RNA interference library, all known open reading frames of the human genome were screened in this new assay system. The "knock down" of genes, and consequently gene products required for prion neurotoxicity, conferred resistance to the neurotoxic effects of the prion protein 106–126 fragment. Eighty not previously described gene targets were identified. Individual genes of interest were re-screened using both retroviral and lentiviral-based expression vectors specifically targeting the gene of interest identified in the initial screen. Further in vitro validation was performed on one of the library positives, E3 ubiquitin-protein ligase, Ube2cbp, that confirmed reduced mRNA and associated reduced protein levels correlated with resistance to toxicity and suggested this protein may be involved in prion pathogenesis. To validate this finding in an in vivo model, immunohistochemistry was performed on scrapie-infected mouse brains. Increased levels of Ube2cbp

were shown to directly correlate with detection of PrPsc. Taken together these results implicate Ube2cbp as a mediator of prion neurotoxicity and demonstrate the utility of this novel neurotoxic assay for screening drug or genetic libraries in search of factors involved in prion pathology.

HD.47: Characterization of truncated forms of cellular prion protein under inflammatory and demyelinating stress condition by proteomic analysis of the spinal cord

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Keywords: prion protein, inflammation, demyelination, fragmentation, dimerization, EAE, prion diseases

Cellular prion protein (PrP^c) is a ubiquitous protein, shown to influence the inflammatory responses by modulating phagocytosis in macrophages[1] as well as T cells activation[2] and is a suggestive risk factor for prion diseases.[3] However PrP form(s) involved in this process has not yet been identified. In the present study we aimed to characterize PrP^c isoforms under inflammatory and demyelinating condition in the spinal cord by using EAE mice model. The detailed expression pattern of PrP^C was analyzed by quantitative one-dimentional (1-DE) and two-dimentional (2-DE) western blots after using series of epitopes specific PrP^C antibodies. The expression pattern of PrP^C showed significant fragmentation and dimerization of PrP^C in the mice under long-term (chronic) inflammatory and demyelinating stress conditions in the spinal cord. Furthermore, we analyzed the specific fragmented isoform of PrP^C by using 2-DE, in-gel digestion, and identified by Q-TOF MS/MS analysis. Together, these results indicate that long-term (chronic) inflammatory and demyelinating stress condition leads to the fragmentation and dimerization of PrP^C and ultimately this might give clues for the understanding of inflammatory processes in CNS in general and in prion diseases in particular including Creutzfeldt-Jakob disease.

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HD.48: Amino acid differences between mouse and human α-synucleins influence pathogenicity

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Alpha-synuclein (SNCA) is linked to both rare familial forms of Parkinson disease and to the common sporadic form. Human A53T mutant SNCA was the first to be associated with a familial form of Parkinson, but in most vertebrates, the wild type residue at position 53 is threonine. Besides the difference at residue 53, mouse and human SNCA differ by 6 of 140 amino acids. We hypothesized that one or more of these amino acid differences protects against the presence of the pathogenic Thr53 in mice.

To determine what effect differences between mouse and human SNCA might have on the proteins' properties, we generated expression constructs in which human amino acids were individually substituted into mouse Snca. Controls included mouse and human WT SNCAs, and the human A53T mutant. Stably transfected SH-SY5Y cell lines were generated. None of the variants increased cell death, and in none of our assays was any Snca variant's properties identical to that of human A53T SNCA.

As the SH-SY5Y results were inconclusive, we tested our variants in yeast. We also made one doubly substituted variant. In a galactose-inducible yeast expression system, using a low copy number vector, four of our mouse variants inhibited yeast growth. Growth was completely inhibited by human A53T SNCA and the doubly mutated mouse Snca variant, and one singly substituted Snca inhibited growth nearly as well. Three variants, and human and mouse wild type did not inhibit growth. We tested the two Snca variants most pathogenic in yeast in mammalian brain, along with mouse WT Snca and human A53T SNCA. Purified Snca proteins were used to generate fibrils in vitro, and after sonication were injected into brains of PrP promoter-regulated A53T SNCA cDNA transgenic mice. One singly substituted variant caused a spinal cord synucleinopathy as quickly as did A53T human SNCA. The same transgenic mice injected with fibrils of mouse wild type Snca had a lifespan significantly longer than those injected with fibrils generated from the other SNCA variants, though they did develop the synucleinopathy. Thus mouse Snca and our variants can misfold in vitro to form a pathogenic template that triggers propagation of pathology through the nervous system from the injection site. We hypothesize that differences in pathogenicity in yeast may reflect differences in the propensity of different Snca species to adopt the misfolded conformation in vivo, and we have identified amino acids that may influence that propensity.

HD.49: Intercellular propagated misfolding of wild-type Cu-Zn superoxide dismutase

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Amyotrophic lateral sclerosis (ALS) is caused by the degeneration of motor neurons in the brain, brainstem, and spinal cord, resulting in progressive paralysis of the limbs, and the muscles of speech, swallowing, and respiration. ALS is predominantly sporadic, but associated with heritable genetic mutations in 5–10% of cases, including those in Cu/Zn superoxide dismutase (SOD1), a free-radical scavenger enzyme. Despite the profusion of functionally diverse genes implicated in FALS and SALS, clinical and pathological similarities between all forms of ALS suggest the existence of a common pathogenic pathway that could be united by a single gene/protein. One of the mechanisms by which a mutant or wild-type (Wt) protein can dominate pathogenesis of phenotypically diverse diseases is by propagated protein misfolding, such as that underpinning the prion diseases, which has been increasingly implicated in other neurodegenerative and systemic disorders.[1] A role for propagated protein misfolding in ALS is supported by the prion-like spatiotemporal progression of disease through the neuroaxis; however the driver of this mechanism remains a mystery. We previously showed that misfolded SOD1 can induce misfolding of natively structured wtSOD1 in a physiological intracellular milieu, in a manner consistent with a direct protein-protein interaction, and that SOD1 misfolding can be transmitted to endogenous human wild-type SOD1 (HuWtSOD1) intracellularly.[2] Our most recent investigations show that misfolding of HuWtSOD1 can be propagated between HEK293 cell cultures through culture via serial passages. Intercellular transmission requires HuWtSOD1 substrate in recipient cells, and can be diminished by both neutralizing antibodies specific to misfolded SOD1, and ultracentrifugation, revealing that the transmission 'particle' is a relatively large. In our attempts to identify this particle, we demonstrate that misfolded SOD1 can be released from cells both on the surface of exosomes, or as aggregates from dead cells. Propagation of HuWtSOD1 misfolding is thus a candidate for molecular pathogenesis of ALS, perhaps generalizable to other wild-type proteins in sporadic neurodegenerative diseases; identification of this prion-like process may prompt novel targeted therapies for ALS and other neurodegenerative diseases.

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HD.50: Manganese exposure induces release of α-synuclein and promotes exosome mediated cell to cell transmission of synuclein aggregates in neurotoxicity models

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Chronic manganese exposure is a well-known occupational and environmental hazard considered to be a potential risk factor for environmentally linked Parkinsonism. αSynuclein (αsyn) is a major presynaptic protein in the CNS, and aggregation of αsynuclein has been implicated in the pathophysiology of Parkinson disease. Since αsyn protein has multiple divalent metal binding sites, we hypothesize that chronic manganese exposure may interact with αsyn to promote protein aggregation and propagation and thereby contributing to dopaminergic neurotoxicity. Previously, we showed that αsyn protects dopaminergic neuronal cells against metal neurotoxicity during early exposure. In the present study, we further characterized the effect of long-term manganese exposure on αsyn metabolism. Immunocytochemistry and inclusion body specific florescent probe studies suggest that prolonged exposure to 300 μM manganese causes aggregation of the human αsyn protein while immunoblots indicate time dependent accumulation of soluble oligomer protein in αsyn expressing N27 dopaminergic cells. To investigate the underlying mechanisms of neurotoxicity caused by possible cell-secreted αsyn species, we have generated human WT αsyn expressed GFP tagged MN9D dopaminergic neuronal cells. Studies conducted with MN9D cells showed that αsyn is secreted into extracellular media following manganese exposure, in a time-dependent manner. Further characterization of condition media by electron microscopy and biochemical analysis revealed that an increased number of exosomes in Mn-treated samples and secreted αsyn is present in these exosomes. In functional studies, we demonstrated that exosomes released during manganese treatment can induce neuroinflammatory responses in primary murine microglial cultures as determined by increased IBA-1 and iNOS expression and increase pro-inflammatory cytokines such as IL-1β, IL-6 and IL12. Collectively, these results demonstrate that prolonged manganese exposure of dopaminergic neuronal cells promotes α-synuclein protein aggregation, stored in exosomal vesicles and secreted into extracellular milieu. Once secreted exosomal vesicles containing αsyn evoked

proinflammatory response in microglia cells, which may contribute to propagation of protein aggregation and progression of neurodegeneration.

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HD.51: Anti prion molecules and their interaction with amyloid β

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Introduction. Cellular prion protein (PrP^C) has many suggested cellular roles, most noticeably its involvement in prion diseases such as Scrapie, BSE and CJD, where soluble cellular PrP^C is converted into insoluble amyloidal form (PrP^{Sc}). The amyloidogenesis process in prion diseases shares a great deal of similarity with the ones in other neurodegenerative diseases such as Alzheimer disease (AD) and Parkinson disease etc. In addition, recent studies clearly showed that PrP^C itself is directly involved in AD by interacting with Aβ peptides and β-secratase (BACE-1). Therefore, it is hypothesized that compounds that exhibit a curative effect on prion disease model could also show promise as therapeutics for Alzheimer Disease.

This work concerns biophysical characterization of the interactions between Aβ, BACE and PrP^C using a variety of techniques including: Atomic Force Microscopy (AFM), fluorescence aggregation studies and Surface Plasmon Resonance (SPR). This leads to the investigation of the effects of a family of novel and potent antiprion compounds and their disruptions to these interactions.

Results. Aβ peptide was expressed in *E.coli* and used in Thioflavin T assays of Aβ aggregation. The results have shown that compounds increase rates of Aβ aggregation but do not ultimately prevent it. Complimentary work using Atomic Force Microscopy has revealed that what appears to be happening in the test tube with Thioflavin T and Aβ in the presence of various compounds does not indicate the true differences in morphology of the protein. SPR was used to investigate the binding of the compounds to the proteins. Compounds have also been found to affect BACE activity at specific concentrations. Finally dosing of Aβ into cell media at different stages of aggregation reduces the effectivity of the compounds in the SMB cell line model.

Conclusion. The results show the compounds' involvement with the enzyme BACE and their effects on the aggregation of the Aβ peptide. Media containing Aβ affects the EC_{50} of the compounds in the SMB cell line model by at least one order of magnitude. Work is currently on going to determine whether this affect on EC_{50} is due to Aβ affecting bioavailability of the drug through its interaction with Aβ, or whether Aβ is being processed in a similar way to PrP increasing burden on the proteosomal pathway that the drugs are believed to target.

HD.52: PrP^C controls via PKA the direction of synaptic plasticity in the immature hippocampus

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The non-pathogenic form of prion protein, PrP^C, is a conserved glycoprotein ubiquitously expressed in almost all tissues, particularly in the brain, where it can be converted into its abnormally folded, aggregated isoform PrP^{Sc} to cause neurodegenerative diseases.

Interestingly, PrP^C is developmentally regulated and in the hippocampus it parallels the maturation of mossy fibers (MF), the axons of granule cells in the dentate gyrus. Moreover, its predominant synaptic localization suggests a crucial role of this protein in synaptic signaling. Previous studies aimed at characterizing PrP^C-deficient mice ($Prnp^{0/0}$) have revealed only mild behavioral changes, including impaired spatial learning, associated with alterations in long-term potentiation.

Here, we tested the hypothesis that, at immature MF-CA3 synapses, which during the first week of postnatal life are mainly GABAergic, PrP^C interferes with synaptic plasticity processes. To this aim, correlated network activity such as GDPs, a hallmark of developmental networks, was used to trigger the stimulation of granule cells in the dentate gyrus in such a way that GDPs coincided with afferent inputs. While in WT animals the pairing procedure induced a persistent increase of MF-$GABA_A$-mediated postsynaptic currents (GPSCs), in $Prnp^{0/0}$ mice it caused a long-term depression (LTD). In WT animals, the induction of LTP was prevented when the calcium chelator BAPTA was loaded into the postsynaptic cell or when the pairing procedure was performed in voltage clamp mode. In these conditions, LTD instead of LTP was revealed. In WT animals, LTP was also blocked when the postsynaptic cell was loaded with the PKA inhibitor PKI suggesting that cAMP-dependent PKA in the postsynaptic neuron is involved in pairing-induced synaptic potentiation. Furthermore, the PKA activator forskolin increased the frequency and amplitude of miniature GPSCs. The increase in amplitude of mGPSCs was blocked by loading the postsynaptic cell with the PKA impermeable inhibitor PKI, indicating a postsynaptic site of action. In contrast, in $Prnp^{0/0}$ mice, forskolin failed to enhance the amplitude of miniature currents suggesting an impairment of PKA activity in postsynaptic neurons. In $Prnp^{0/0}$ mice, LTD was presynaptic and was prevented by the selective GluK1 antagonist UBP 302 or by the phospholipase C inhibitor U73122, suggesting that it relied on G protein-coupled GluK1 receptors. Postsynaptic infusion of a constitutively active isoform of PKA catalytic subunit (Cα) into CA3 principal cells of $Prnp^{0/0}$ mice caused a persistent synaptic facilitation and occluded LTP induced by subsequent pairing. These data suggest that PrP^C plays a crucial role in regulating via PKA synaptic plasticity and information processing in the developing hippocampus.

HD.53: Transmission of amyloid-β misfolding: Profile of aggregation using Alzheimer samples harboring different types of Aβ deposits

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Objectives. Accumulation of misfolded Abeta is one of the main pathological features of Alzheimer disease (AD). We and others have demonstrated that Abeta pathology can be induced by a prion-like mechanism in animal models after intra-cerebral administration of Abeta aggregates. Our recent experiments suggest that Abeta pathogenesis can also be induced by blood transfusions. These findings urge for additional experiments directed to understand the nature of the transmissible agent and the role that spreading and transmission of Abeta aggregation may play in the etiology and progression of AD.

Materials and Methods. Transgenic mice models of AD were intracerebrally challenged with brain samples from different cases of AD. Additionally, we injected brain samples from patients affected by Mild Cognitive Impairment (MCI) and Non-Demented individuals with Alzheimer Disease Neuropathology (NDAN). Animals were sacrificed several months after injection. Tissues were collected for biochemical and histological analyses.

Results. The prion-like propagation of Abeta misfolding was reproduced in two different mouse models of AD. We measured the amount, brain distribution, morphological characteristics and profile of Abeta aggregates induced by intracerebrally injection into mice. Injection of brain extracts from different AD patients induced Abeta aggregation differently, suggesting that this phenomenon can be dictated by the misfolded seed. Brain extracts from individuals classified as MCI and NDAN also promoted amyloid deposition and showed particular profiles of Abeta aggregation in the brain of experimental subjects. These results suggest that seeding activity is present in the brain before the appearance of the clinical symptoms of the disease.

Conclusion. Our findings, together with previously published reports, suggest that some aspects of AD pathology might be transmissible. However, additional experimental and epidemiological studies are necessary to unveil the role of these processes in the etiology of the disease in humans. These results may contribute to understand the mechanisms implicated in the initiation of Abeta pathology and therefore be useful to develop new therapeutic strategies for the prevention and treatment of this devastating disease.

HD.54: Studies on efficient propagation and affecting factors in heparinized cell-protein misfolding cyclic amplification (PMCA) of variant Creutzfeldt-Jakob disease (vCJD) prion spiked into plasma

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Background. To prevent the iatrogenic spread of variant Creutzfeldt-Jakob disease (vCJD) between humans via blood transfusion or biologicals, highly sensitive in vitro screening tests are necessary. Protein misfolding cyclic amplification (PMCA) is a candidate for such a test. Although we reported that PrP^{Sc} in 10–10 diluted vCJD brain was detected by heparinized cell-PMCA,[1] plasma has been reported to inhibit the PMCA reaction.[2] Therefore, we investigated cell-PMCA conditions, which permit vCJD prion amplification under the presence of plasma, and plasma factor, which affect PMCA efficiency.

Materials and Methods. Cell-PMCA of vCJD was performed by adding various final concentrations of pooled normal plasma (1–50%), citrate-phosphate-dextrose (CPD, anti-coagulant), albumin, IgG or pooled plasma treated with ion-exchanger. The optimal heparin concentration was studied at each plasma concentration. Serial 100-fold dilutions of vCJD brain were prepared in plasma and were subjected to multi-round PMCA by keeping the final plasma concentration at 40% at each round.

Results. When 1 to 50% pooled plasma were added to heparinized cell-PMCA, amplification efficiency showed double-peaked profile; it was comparable to that without plasma at 1 and 40% final pooled plasma concentrations, but decreased at the other concentrations. PMCA reaction was inhibited when CPD was added at equivalent concentrations included in 20% plasma or over, but not when albumin or IgG was added at any concentration tested. The passed fractions of plasma through an anion exchanger inhibited the PMCA reaction, but that through a cation exchanger didn't. When the final plasma concentration was at 30% or over, optimal heparin concentration was higher than 100 μg/mL, which is optimal without plasma. Finally, multi-round cell-PMCA was performed and PrP^{Sc} in 10–10 dilution of vCJD brain was detected by the fifth round, of which the detection limit is comparable to that without plasma.[1]

Conclusion. We found the specific condition where vCJD prion can be amplified efficiently even under the presence of plasma by cell-PMCA. Our date suggest that plasma contains not only PMCA inhibitors but also stimulator, which are unknown at present.

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HD.55: Exploring physical and chemical factors affecting RT-QuIC

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Background/Introduction. Real-time quaking-induced conversion (RT-QuIC) assay has been reported as a highly sensitive and specific method for testing the existence of prion protein in brain homogenate of the 263K hamster prion disease model and human CSF from sporadic CJD patients.[1-3] This technique has the potential to provide a routine ante-mortem diagnostic test for prion disease based upon the presence of the etiologic agent. In order to standardize the method in our laboratory, the effects of several physical and chemical factors on the sensitivity, specificity, and reproducibility of the assay have been assessed.

Materials and Methods. Full-length recombinant hamster prion protein (rPrP) was purified as previously described[1,2] with some modifications. Denatured rPrP was refolded on an HPLC system under low pressure overnight (16 h) in phosphate buffer at pH 8.0 and a flow rate of 0.6 ml/min in. After refolding, the column was connected to an FPLC system for rPrP elution in 100 mM phosphate buffer containing 500 mM imidazole, pH 5.8. Following elution, the rPrP fractions were diluted in 1/3 volume of dialysis buffer (10 mM phosphate buffer, pH 5.8) in order to prevent precipitation. The eluted solutions were filtered with a 0.22 μm syringe filter before dialysis. The dialysed rPrP fractions were re-filtered before protein quantitation at 280 nm. Various physical and chemical parameters were tested over several RT-QuIC assays.

Results. The sensitivity and reproducibility of the RT-QuIC assay were affected by several factors. The handling and storage of the rPrP substrate were critical. The use of spin filters to increase the concentration of rPrP or prolonged storage of the substrate at both 4°C and -80°C negatively affected assay performance. The salt concentration of the substrate buffer was also of critical importance. Previously reported salt concentrations of ≥ 130 mM[3] caused spontaneous aggregation of rPrP and prevented signal detection. When using freshly made rPrP in the absence of salt, signal levels peaked at a 10–2 dilution of 263K hamster brain homogenate seed and remained consistently positive up to a 10–6.dilution. The use of a sheep/hamster chimera rPrP substrate is also being explored. Once optimal conditions for this substrate have been defined, the relative sensitivity and specificity of the two recombinant proteins will be assessed.

Conclusion. Laboratory-specific optimal conditions are necessary for a consistent RT-QuIC assay result. Harmonisation and standardisation of RT-QuIC between laboratories will be challenging.

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HD.56: A cation exchanger specifically enhances in vitro amplification of variant Creutzfeldt-Jakob disease prion despite its weaker captureing ability

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Introduction. Ultra-sensitive detection methods for variant Creutzfeldt-Jakob disease (vCJD) prion have been developed recently. Protein misfolding cyclic amplification (PMCA) is one of them, and we established cell-PMCA, where prion in vCJD brain is amplified by the magnitude of approximately 1,000-fold (Yokoyama et al., Prion 2011). Aiming at concentrating prion prior to PMCA and/or enhancing PMCA efficiency, we assessed effects of particles on PMCA.

Materials and Methods. We examined binding capacities to prion and effects on PMCA efficiency of 31 types of particles including resins for size exclusion, ion exchange, or hydrophobic chromatography, and inorganic compounds such as metals. vCJD brain homogenates (vCJD-BH) were spiked into pooled human plasma, and incubated overnight after adding various particles. Particles and supernatants were subjected to proteinase K digestion followed by western blotting. Second, particles were incubated with serial dilutions of vCJD-BH in plasma, removed and subjected to cell-PMCA. PMCA products were digested with proteinase K, followed by western blotting to analyze amplification efficiency.

Results. Five out of six anion exchangers and only Fractogel SO_3^- out of seven cation exchangers absorbed > 90% prion in vCJD-BH spiked plasma, but the rest of particles did not. Surprisingly, these good absorbent resins did not enhance PMCA efficiency. Regardless of the presence or the absence of particles, prion in 10^{-6} or 10^{-7} vCJD brain diluted in plasma was detected after a single round of cell-PMCA, except for SP Sepharose with which prion in 10^{-9} or 10^{-10} vCJD-BH spiked

plasma was detected, and with which total reaction periods to detect prionin 10^{-10} vCJD-BH was reduced from 20 d to 5 d. Interestingly, absorbing ability of SP Sepharose was not so high; less than 50%. Finally, when SP Sepharose was used in PMCA of 263K, strong inhibition was observed, indicating that effect of SP Sepharose depends on prion strains.

Conclusion. Cell-PMCA with SP Sepharose significantly increased the magnitude of amplification efficiency, resulting in three quarters reduction of required reaction time to detect very minute amounts of vCJD prion.

HD.57: Functional analysis of YKL100C, a yeast presenilin/signal peptide peptidase homolog, reveals features similar to γ-secretase-independent action of presenilin 1

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γ-secretase, the enzyme responsible for generation of Abeta peptide implicated in Alzheimer Disease, is comprised of at least 6 possible combinations of 4 subunits, and mediates intramembrane cleavage of more than 60 protein substrates. Studies are underway in our laboratory to reconstitute human gamma-secretase in yeast, as a system to enable functional analysis of individual enzyme complexes toward individual substrates. Concurrent functional studies of hypothetical protein YKL100C, a yeast homolog of presenilin 1, the catalytic subunit of gamma-secretase are also being performed. YKL100C bears the sequence hallmarks of aspartyl proteases involved in regulated intramembranous proteolysis (I-CliPs, or intramembranously cleaving proteases) and displays signal peptide peptidase (SPP) activity in vitro. N-linked glycosylation studies revealed that only the first three of five sites were occupied, and that activity was glycosylation independent. However, SPP activity was dependent on retention of the Asn residues of the last two N-glycosylation sequons. Engineered N-glycosylation experiments supported an SPP-like membrane orientation for the catalytic transmembrane (TM) domains of YKL100c (rather than a presenilin-like orientation). YKL100C undergoes proteolytic cleavage reminiscent of presenilin activation, and epitope-tagging along with antibody mapping has permitted localization of cleavage sites within YKL100C. YKL100C is non-essential in yeast, but deletion led to vacuolar acidification and vacuolar morphology alterations and these could be reversed by introduction of wildtype YKL100C, by catalytically-inactive YKL100C, and also by wildtype and FAD mutant versions of presenilin 1 (PS1). Further exploration of a protease-independent role for YKL100C are underway, focusing on intracellular trafficking and expression of two candidates, Vph1 (vacuolar ATPase, V_0 subunit) and Pma1 (plasma membrane H^+ ATPase). In summary, biochemical characterizations of the protein encoded by the yeast YKL100C locus revealed enzymatic and membrane topological similarities to SPP, while cell biological studies suggest YKL100C bears features similar to the γ-secretase independent functions of presenilin.