Prion 2016 Oral Abstracts

O-01: Folding and misfolding pathways of prion protein

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The mechanism by which a random-coil polypeptide folds into the native structure may be critical for the regulation of misfolding diseases. However, how the folding pathway is related to the misfolding pathway remains unclear. Our recent study demonstrated that the native folding pathway of prion protein (PrP) involves at least 4 independent species, including native state (N), unfolded state (U), and 2 types of partially folded states (A and I) (Honda et al., Structure, 2015). Interestingly, one of the partially folded states (A state) readily formed a misfolded aggregate whose rates were strongly correlated with the initial population of the A state (Honda et al., J Biol Chem, 2014). This observation indicated that the formation of the A state may be the initial step in the misfolding pathway. We characterized the structure of the A state using circular dichroism, hydrogen/deuterium exchange coupled with NMR, etc. and found that the Strand 1-Helix 1-Strand 2 segment was completely unfolded in the A state, whereas the Helix 2-Helix 3 segment retained a native-like helical structure. Our studies revealed how the native structure is altered during the early stage of misfolding and how the misfolding pathway is related to the native folding pathway of PrP.

O-02: HET-2s, an engineered, 4-rung \(\beta\)-solenoid protein as a model for the structure of PrP\(^{Sc}\)

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The high-resolution structure of PrP\(^{Sc}\) has eluded experimental determination due to its insolubility and propensity to aggregate. Nevertheless, the repeating nature of PrP\(^{Sc}\) aggregates (e.g. amyloid fibrils) has allowed the collection of lower-resolution structural data via X-ray fiber diffraction, electron cryomicroscopy, and other techniques. X-ray fiber diffraction from PrP\(^{Sc}\) amyloid fibrils indicated a repeating unit of 1.92 nm per molecule, based on a series of meridional diffraction signals at 0.48, 0.64, and 0.96 nm. The diffraction data established that the structure of PrP\(^{Sc}\) contains a 4-rung \(\beta\)-solenoid core.\(^1\) Furthermore, electron cryomicroscopy measurements determined a molecular height range of 1.91 - 1.97 nm for each monomer of PrP\(^{Sc}\), while confirming the presence of 0.48 nm cross-\(\beta\) structure in individual amyloid fibrils. Therefore, electron cryomicroscopy independently confirmed the 4-rung \(\beta\)-solenoid architecture of PrP\(^{Sc}\).

The structure of the prion domain of the fungal HET-s prion protein was determined by solid-state NMR spectroscopy to contain a 2-rung \(\beta\)-solenoid structure.\(^2\) This structural similarity made the prion domain of HET-s an ideal target to engineer a conformational mimic for the structure of PrP\(^{Sc}\). By doubling the coding...
sequence of the HET-s prion domain, connected by a flexible linker, we set out to engineer a 4-rung \(\beta\)-solenoid variant of the HET-s prion domain, termed HET-2s. We constructed an atomic-resolution model for HET-2s using the NMR-derived structure\(^2\) and the SWISS-MODEL server. The stability of the model was subsequently verified by molecular dynamics simulations.

Recombinantly expressed HET-2s was purified and fibrillized under the same conditions as regular HET-s. Negative stain and cryo low-dose electron microscopies were used to characterize the HET-2s construct and found it to conform to the same structural parameters as regular HET-s amyloid. Therefore, we conclude that the structure of HET-2s is indeed a 4-rung \(\beta\)-solenoid, as intended / designed. Future experiments will be conducted to compare the structures of HET-2s and PrP\(^{Sc}\), both differences and similarities will provide stimulating insights into the remaining mysteries of PrP\(^{Sc}\).

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O-03: Prion protein deficiency causes diverse proteome shifts in cell models that escape detection in brain tissue

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A popular method for studying the function of a given protein is to generate and characterize a suitable model deficient for its expression. For the prion protein (PrP), best known for its role in several invariably fatal neurodegenerative diseases, a natural choice, therefore, would be to undertake such studies with brain samples. We recently documented the surprising observation that PrP deficiency caused a loss or enhancement of NCAM1 polysialylation, dependent on the cell model used. To identify possible causes for this disparity, we set out to systematically investigate the consequence of PrP deficiency on the global proteome in brain tissue and in 4 distinct cell models, in which PrP-related phenotypes had previously been described. Here we report that PrP deficiency causes robust but surprisingly divergent changes to the global proteomes of cell models but has no discernible impact on the global brain proteome. Among >1,500 proteins, whose levels were robustly quantified and compared in all wild-type and PrP-deficient models, members of the MARCKS protein family exhibited pronounced, yet cell model-dependent changes to their steady-state levels in PrP-deficient cells. Follow-up experiments revealed that PrP collaborates with members of the MARCKS protein family in its control of NCAM1 polysialylation. Our data suggest that careful investigations in cell models, rather than mouse tissue, are the way forward for elucidating the molecular function of PrP.
O-04: Protein folding activity of the ribosome: Key player in yeast prion propagation

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Using successive screenings for drugs able to cure the unrelated [PSI+] and [URE3] yeast prions as well as PrPSc mammalian prion, we showed some mechanisms controlling prion propagation to be conserved from yeast to humans. These screenings identified several new antiprion compounds: 6-aminophenanthridine (6AP), guanabenz (GA, already used in hypertension) and imiquimod (IQ, a TLR7 agonist), all active against yeast and mammalian prions in vitro and in vivo. 6AP and GA were independently shown to interact with specific domain V nucleotides of the large rRNA of the large 60S ribosome subunit. Domain V of the large rRNA (23S in E. coli, 25S in S. cerevisiae, 28S in metazoa) has 2 enzymatic activities, i) a peptidyl transferase activity and ii) a poorly characterized protein folding activity, PFAR: Protein Folding Activity of the Ribosome. PFAR was characterized by in vitro refolding experiments showing that domain V of the large rRNA from bacteria, yeast or drosophila assist protein folding of various denatured protein substrates. PFAR was mainly studied in vitro and its biological role remains unclear. 6AP and GA are the first specific competitive inhibitors of PFAR not affecting protein synthesis. As 6AP and GA were first identified as inhibitors of yeast and mammalian prions, we explored the link between PFAR and [PSI+] prion in yeast. We demonstrated that PFAR is involved in the propagation of [PSI+]. We also showed that PFAR and Hsp104p partially compensate each other for [PSI+] propagation: reduced Hsp104p activity, which is deleterious for [PSI+] propagation, is compensated by the enrichment in PFAR activity, and vice versa. PFAR is thus an evolutionarily conserved cell component implicated in the prion life cycle, and as such represents a potential new therapeutic target to treat other amyloid-based diseases such as Alzheimer’s and Huntington’s disease.

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O-05: A local conformation of natively disordered yeast prion monomer determines interspecies prion transmissibility

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Prions are infectious agents that cause fatal neurodegenerative disorders in mammals. While prion transmission between different species is very limited, a molecular mechanism underlying the species barrier remains largely unclear. Here, we used the yeast prion Sup35NM as a model protein from 2 highly divergent yeast species, S. cerevisiae (SC) and K. lactis (KL), and exploited several biophysical and yeast genetics approaches to investigate how prion monomer dynamics affect the interspecies prion transmission.

First, we used nuclear magnetic resonance (NMR) spectroscopy to assess the molecular motions of SC and KL Sup35NM monomers. A variety of NMR analyses revealed that only a small segment of the protein showed a remarkably different protein motion between the 2 Sup35NM monomers. To examine whether this difference plays a role in prion transmission between the 2 species, we prepared a SC-based Sup35NM chimera protein by replacing this short segment with corresponding amino acid residues in KL Sup35NM and performed a cross-seeding assay using an amyloid-specific dye, thioflavin T. Strikingly, the SC chimera monomer interacted with KL Sup35 amyloid as effectively as KL Sup35NM monomer. Furthermore, we found that the replacement of several amino acids was sufficient to confer the SC Sup35NM protein with a high cross-seeding reactivity toward KL Sup35NM amyloids. The cross-seeding was also confirmed \textit{in vivo} by cytoduction experiments. Finally, we performed NMR analyses in order to examine whether these mutations alter SC Sup35 monomer dynamics. Surprisingly, the mutations in the SC Sup35NM chimera were sufficient to induce the formation of the local conformation that KL Sup35NM adopts.

Taken together, the present study revealed that a local conformation of natively disordered Sup35NM is crucial for breaking the prion transmission barrier.

O-06: Prion nucleation and propagation by amyloid $\beta$ in the yeast model

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Amyloid formation is implicated in various mammalian and human diseases, and many amyloids are suspected to possess transmissible (prion) properties. However, the molecular mechanisms of amyloid formation and propagation are difficult to investigate \textit{in vivo} due to complexity of the human organism. We have established a yeast model for studying the prion properties of mammalian and human proteins. This model employs chimeric constructs, containing the mammalian amyloidogenic proteins.
(or domains) fused to various fragments of a yeast prion protein. Phenotypic and biochemical detection assays, previously developed for prion detection in yeast, enable us to detect prion formation by mammalian proteins. Our approaches allow to distinguish between the initial amyloid nucleation and further propagation of a prion state. By using these approaches, we have investigated prion-like properties of amyloid β (Abeta) peptide associated with Alzheimer disease (AD). We have demonstrated that mutations blocking formation of cross-β structures prevent prion nucleation by Abeta in the yeast assay, while familial AD mutation D23N increases prion nucleation. We have also shown that mutation K28E disrupting a salt bridge within the Abeta peptide decreases prion nucleation, while restoration of the salt bridge by a reciprocal mutation at the position 23 partially restores prion nucleation. These data confirm the role of cross-β interactions and salt bridge in amyloid nucleation by Abeta, and show that the yeast prion nucleation assay provides a relevant model for the investigation of amyloidogenesis by Abeta. We have also developed the yeast assay for Abeta-driven prion propagation and employed this assay to generate and propagate various Abeta-dependent prions in yeast, showing that Abeta can produce a variety of distinct self-perpetuating prion strains. Moreover, we have demonstrated that Abeta-based prion strains can be produced by transfecting yeast cells with in vitro generated Abeta aggregates. Finally, we have investigated effects of some chemicals and chaperones on Abeta-dependent prion nucleation or propagation in yeast. (Supported by grants P50AG025688 from National Institutes of Health and 14-50-00069 from Russian Science Foundation).

**O-07: Oral prion pathogenesis is reduced in the absence of CXCR5-expressing mononuclear phagocytes**

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After oral exposure to prions, the early replication of certain prion strains upon follicular dendritic cells (FDC) in the Peyer’s patches in the small intestine is important for the efficient spread of disease to the brain (termed neuroinvasion). However, little is known of how prions are initially conveyed from the lumen of the gut to the FDC. Our data suggest prions are initially acquired from the gut lumen by M cells in the epithelia overlying the Peyer’s patches. Following their transcytosis by M cells, particles and microorganisms typically exit into the intraepithelial pocket on the basolateral membrane where they are subsequently processed by the underlying leukocytes and lymphocytes. Migratory bone marrow-derived conventional dendritic cells (cDC) are centrally involved in the transport of antigens both within Peyer’s patches and on into the mesenteric lymph nodes. A subset of cDC has also been identified that can migrate into the B cell follicles where FDC reside. We have previously show that in the transient absence of CD11c-positive mononuclear phagocytes (such as cDC) at the time of oral exposure, the accumulation of prions in the Peyer’s patches is impeded and disease susceptibility reduced. However, whether these mononuclear phagocytes convey prions directly to FDC within the B cell follicles of Peyer’s patches is not known. Chemokines play important roles in attracting lymphocytes and leukocytes to lymphoid tissues and controlling their positioning within them. The chemokine CXCL13 is expressed by FDC and modulates the homing of CXCR5-expressing B cells toward them. Certain CD11c-positive mononuclear phagocytes also express CXCR5 and can migrate into B cell...
follicles. We therefore determined whether CXCR5-expressing mononuclear phagocytes convey prions to FDC after oral exposure. Novel compound transgenic mice were created in which CXCR5-deficiency was specifically restricted to CD11c-positive mononuclear phagocytes. When these mice were orally exposed to prions our data show that in the absence of CXCR5 expression in mononuclear phagocytes the early accumulation of prions upon FDC in Peyer’s patches and the spleen was impaired, and disease susceptibility was significantly reduced. Together, these data suggest that after oral exposure CXCR5-expressing mononuclear phagocytes play an important role in the efficient transfer of prions toward FDC within Peyer’s patches. A thorough understanding of the cells and mechanisms involved in the propagation of prions within the gut will help identify novel factors that influence disease susceptibility or targets for intervention.

**O-08: Tau pathology in Creutzfeldt-Jakob disease: Novel insights**

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Creutzfeldt-Jakob disease (CJD) is a transmissible spongiform encephalopathy with deposition of disease-associated prion protein in the brain. While small tau immunoreactive neuritic profiles are readily found in all subtypes, there is a relative paucity of reports on other types of tau pathologies in CJD. The objective of our study was to investigate the pattern of tau pathology in CJD. We performed a comprehensive mapping of tau pathologies using the phosphorylated tau antibody AT8 in sporadic CJD (sCJD) and a cohort of genetic CJD (gCJD) cases. We conducted a semiquantitative analysis of tau immunoreactivities in the entorhinal cortex and 6 hippocampal subregions. We examined 83 consecutive cases of sporadic CJD (sCJD). 20 cases showed only tiny dots of AT8 immunoreactivity in the neuropil with or without concomitant non-tau neurodegenerative disorders. From the remaining 63 cases, 52 showed tau pathology in the medial temporal lobe and further 11 widespread tau pathologies compatible with features of primary tauopathies or the gray matter type of aging-related tau astrogliopathy (ARTAG). Prominent ARTAG was observed also in 2 out of 3 V203I gCJD cases. In 30/52 (57.7%) of the sCJD cases the distribution of tau pathology followed the Braak stages of neurofibrillary pathology. However, in 22/52 cases (42.3%) the subregional distribution of tau pathology was significantly different and deviated from the patterns compatible with Braak staging. They were characterized by lower phospho-tau immunoreactivity score in the entorhinal cortex as compared to the subiculum, the dentate gyrus or CA4 region of the hippocampus. Although the overall frequency of neuronal and glial tau pathologies is not unusually high in CJD, the pattern of hippocampal tau pathology often deviates from the stages of Braak. Our study indicates that primary tauopathies and ARTAG can be also observed in human prion disease.

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O-09: Creutzfeldt-Jakob disease prion propagation in human iPS cells-derived astrocytes

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The absence of a human cell culture model, in which human Creutzfeldt-Jakob disease (CJD) prions replicate, has frustrated CJD research for over 4 decades, and represents a major obstacle in the discovery of effective therapeutics. The mechanisms of neurodegeneration responsible for prion diseases are not completely understood, but increasingly, interactions between neurons and glia are thought to be involved. One of the hallmark's of the prion disorders is reactive astrogliosis, and some in vivo experiments argue that the PrP\textsupersc{Sc} pathogen replicates both in astrocytes and neurons.

Based on these findings, we developed a human cell system for propagating CJD prions in vitro using human iPS cells differentiated into functional astrocytes. Astrocytes were transiently exposed to sporadic or variant CJD brain homogenates and further cultured in fresh media for several days. The accumulation of nascent human (Hu) PrP\textsupersc{Sc} in these cultures was measured by immunoblotting and immunocytochemistry. Human astrocytes were able to support CJD prion replication but required matching methionine/valine residue 129 encoded by the PRNP gene with the HuPrP\textsupersc{Sc} in the prion inoculum. Importantly, CJD prion replication required differentiated astrocytes since proliferating human astrocyte precursor cells were unable to support PrP\textsupersc{Sc} replication.

Our results suggest that replication of CJD prions requires a post-mitotic human cellular phenotype such as the astrocyte. Furthermore, the inocula and host cells must be matched for the codon 129 PRNP polymorphism as noted above. We hypothesize that astrocytes contribute to prion-induced neurodegeneration in a non-cell autonomous manner, either by impairment of astrocytic neuroprotective functions or generating neurotoxic factors in response to prion infection.

To our knowledge, this is the first demonstration that normal, functional and non-transformed human cultured cells are susceptible to infection with human PrP\textsupersc{Sc} prions in vitro. Our human iPS cells-derived neural cell model could provide a relevant in vitro model system for studying mechanistic neurotoxicity and a powerful tool for drug discovery for PrP\textsupersc{Sc} prion diseases and other prion-related neurodegenerative diseases.

O-10: Role of Tunneling Nanotubes (TNTs) in intercellular spreading of prions and other protein

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Neurodegenerative diseases (NDs) such as Prion disease, Alzheimer disease (AD), Parkinson disease (PD) and Huntington’s disease (HD) are part of a larger group of protein misfolding disorders (PMDs) characterized by the progressive accumulation and spreading of
protein aggregates of different sizes - oligomers, protofibrils or fibrils-, which ultimately can assemble into extracellular amyloid deposits and/or intracellular inclusions. The best-characterized example of PMDs is prion diseases, which are caused by the conversion of the normal form of the prion protein (PrPC), to a misfolded form (PrPSc) through template conformation changes. Like in Prion diseases, misfolded forms of α-synuclein, tau, Abeta and Htt proteins associated with AD, PD and HD can be transmitted experimentally in cellular and in animal models where it can act as ‘seeds’ to recruit the endogenous protein into aggregates (seeding process). However, the mechanism of intercellular transfer is still obscure.

We have recently described a novel mechanism of PrPSc transmission through Tunneling Nanotubes (TNTs). TNTs are actin-based fine protrusions connecting sparse cells in culture and represents a novel mechanism of cell-to-cell communication. We showed that TNT are mediating both exogenous and endogenous PrPSc transfer between infected and naïve mouse neuronal cells and between bone-marrow dendritic cells and primary neurons. Using co-culture systems between primary infected astrocytes and granule neurons, we provide direct evidence that prion-infected astrocytes can disseminate prion to neurons through intercellular TNT-like connections, thus suggesting that astrocytes may contribute to the progression of the disease. Finally, our most recent data show that α-synuclein fibrillar assemblies transfer inside lysosomal vesicles in TNTs and they are able to seed the misfolding of the soluble protein.

Based on these data we propose that TNTs contribute to the progression of the pathology of neurodegenerative diseases associated with the spreading in the brain of misfolded protein assemblies.

**O-12: PrPSc in the skin of CJD patients**

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Sporadic Creutzfeldt-Jakob disease (sCJD) is the most common form of human prion disease, transmissible by neuroinvasive iatrogenic routes. Prion infectivity is abundant in sCJD brains, but it is less detectable in peripheral tissues and has never been reported in skin. Although equivocal, several epidemiological studies have ascribed the low but statistically significant sCJD risk to various surgeries involving skin. Moreover, the definitive diagnosis of sCJD is virtually completely dependent on examination of the brain obtained by autopsy or biopsy, which always has been a challenge. Here, we have tested for abnormal CJD-associated prion protein (PrPSc) in the skin using Western blotting and real-time quaking-induced conversion (RT-QuIC). Skin samples were collected at autopsy or by biopsy from 5 sCJD, one vCJD, and 10 non-CJD patients and subjected to RT-QuIC and Western blotting for PrPSc. We demonstrated that PrPSc and prion seeding activity are present in the skin of CJD patients. Our presentation will illustrate the detailed new findings and discuss the implications raised by this study (Funded by the CJD Foundation, the National Institute of Neurological Disorders and Stroke and others).
O-13: Autopsy validation of second generation RT QuIC for diagnosis and differentiation of human prion diseases: Results from the US National Prion Disease Pathology Surveillance Center

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The development of new in vitro amplification systems - the second-generation real-time quaking-induced conversion (RT QuIC) - allows detecting attogram amounts of pathogenic prion protein (PrPSc) in human cerebrospinal fluid (CSF). We performed second-generation CSF RT QuIC (IQ) tests in a cohort of 2,141 patients with rapidly progressive neurological disorder suspected of prion disease. We determined the diagnostic sensitivity and specificity in 275 cases that underwent autopsy and detailed neuropathological assessment. The diagnostic specificity was 98.5% and sensitivity 89-92% in an autopsy-validated retrospective cohort. In the ongoing autopsy-validated prospective study, the diagnostic specificity thus far is 100% and sensitivity 96%, reflecting a higher proportion of cases with more aggressive disease. The impact of polymorphism in codon 129 genotype and type 1 or 2 of CJD PrPSc on the sensitivity and specificity of RT QuIC was evaluated using detailed neuropathology, molecular typing of brain PrPSc, and sequencing of PRNP gene. The CSF RT QuIC data and genotyping performed together allow to correctly differentiate the sCJD MM1 from sCJD MM2 type with 94% accuracy, and sCJD VV1 from sCJD VV2 type with 80% reliability. The effect of PRNP gene mutations, age, gender, clinical, and pathological phenotype, as well as diseases stage and progression rate are being evaluated in an ongoing investigation. We will discuss the impact of these new prion detection strategies on surveillance of human prion diseases. (Supported by CJD Foundation and funded by the CDC and the National Institute of Neurological Disorders and Stroke).

O-14: Highly sensitive and specific detection of prions in blood of vCJD patients by PMCA

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Transmissible Spongiform Encephalopathies (TSE’s) are a group of fatal infectious neurodegenerative diseases, including CreutzfeldtJakob disease in humans (CJD), bovine spongiform encephalopathy in cattle (BSE), scrapie in sheep and goat, and chronic wasting disease (CWD) in deer. The infectious agent causing these diseases is called prion or PrP scrapie (PrPSc), which is an abnormal conformation of the innocuous cellular PrP (PrPC). Prions are able to self-replicate at expenses of PrPSc and spread in the central nervous system causing neuronal death.

The development of a biochemical assay for the sensitive, specific, early and non-invasive detection of prions in blood of patients affected by prion disease is a top medical priority to increase the safety of the blood supply. The new variant CJD (vCJD), first reported in United Kingdom (UK) after an outbreak of BSE, has already been transmitted from human-to-human by blood transfusion and the number of asymptomatic carriers of vCJD in the UK alone is estimated to be 1 in 2,000 people.
In this study we used the protein misfolding cyclic amplification (PMCA) to analyze samples of whole blood from 14 cases of vCJD and 137 controls, including healthy individuals and patients affected by other neurodegenerative or neurological disorders. As additional controls, we used whole blood, plasma and white cells from sporadic CJD patients. Our results show that we can detect PrPSc with 100% sensitivity and specificity in the vCJD samples tested. Detection was possible in any of the blood fractions analyzed and can be done with as little as few μL of sample volume.

Our findings indicate that PMCA may be useful for pre-mortem non-invasive diagnosis of vCJD and to identify prion contamination of the blood supply. Further studies are needed to investigate the earliest time during the pre-clinical phase of vCJD when PrPSc is detectable in blood.

O-16: Puzzling out the BSE-human transmission barrier

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To date, Bovine Spongiform Encephalopathy (BSE) is the only recognized zoonotic prion causing variant Creutzfeldt Jakob Disease (vCJD) in humans. The major risk determinant for this disease is the human PrP dimorphism (Met/Val) at codon 129 as all tested definite clinical cases are homozygous Met129. Alternative codon 129 genotypes (homozygous Val129 or heterozygous Met129Val) concern about the risk of further vCJD outbreaks in humans carrying these natural PrP variants. In addition, the virulence and the susceptible host range of a prion strain can switch through intermediate species suggesting that the transmission of BSE to other species could be an additional risk factor to humans.

Our transgenic mice expressing human PrP C (HuPrP-Tg) carrying different variants at codon 129 (129Met or 129Val) faithfully mimic the cattle-BSE behavior in humans. While homozygous Met129 mice were susceptible to BSE infection developing a Transmissible Spongiform Encephalopathy with strain phenotypic properties indistinguishable from vCJD, heterozygous Met129Val and homozygous Val129 mice were resistant to the disease transmission even after 2 serial passages and neither PrPres nor infectivity could be detected in their brains as assessed by subsequent passage in transgenic mice expressing bovine PrP C (BoPrP-Tg).

Similarly to cattle-BSE, BSE derived prions from other species (sheep, goat and pig) maintained their transmission ability restricted to the homozygous Met129 mice. Transmission of BSE prions into humanized transgenic mice carrying the Val129 allele was only possible after previous propagation/adaptation in Met129 mice. Even after adaptation, a strong transmission barrier and very long incubation times were observed in homozygous Val129 mice. A similarly high transmission barrier was observed when these mice were inoculated with vCJD isolates from human natural cases.

All this data corroborate the strong influence of the Met129Val human PrP dimorphism in the transmission barrier to BSE/vCJD agents and are of great value in risk assessment of vCJD secondary transmission in humans.

O-17: Acceleration of Abeta brain amyloidosis by peripheral administration of disease associated aggregates

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Amyloid-β (Abeta) deposits in the brain of transgenic animals have been shown to increase
when they are challenged with samples containing Abeta aggregates. Most of these studies have been done by directly inoculating Abeta aggregates in the brain of susceptible mice. In here, we show that brain amyloidosis associated to Alzheimer disease’s Abeta is accelerated after exposing animals to biologically active seeds by different routes of administration. Experiments were done in 2 transgenic mouse models expressing the human version of the Amyloid Precursor Protein and using brain derived and purified/recombinant Abeta seeds as inocula. Pathological changes generated in challenged mice were compared to natural amyloid deposition developed in untreated transgenic animals sacrificed at different ages. Our results show that many of the routes of administration tested accelerated pathological changes in the brain. The type of aggregates generated, as well as their distribution along the brain, were analyzed in all cases. We found a strong correlation between seeded aggregates and blood vessels, suggesting vascular transport of the seeds from the periphery to the brain. We also found that Abeta oligomers were more efficient to spread Abeta deposition than large amyloid fibrils. Our findings suggest that some aspects of Alzheimer disease pathology might be transmissible by relevant routes of administration. Also, this information highlight an important role for peripheral structures in the brain pathology. These results may contribute to understand the mechanisms implicated in the origin and spread of Abeta pathology and be of importance to create therapeutic and diagnostic strategies for disease prevention and treatment.

Transmission of human prion diseases through medical practices resulting in iatrogenic Creutzfeldt-Jakob disease (iCJD) is well documented. The most common procedures causing iCJD included injection of human growth hormone (hGH) derived from cadaveric pituitary glands and human dura mater (hDM) grafting. However, worldwide incidence of iCJD varies possibly depending on hGH and hDM graft formulations. Recently, 2 studies of

**O-18: Amyloid β pathology in iatrogenic Creutzfeldt-Jakob disease: A multicenter study**

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iCJD cases reported prominent presence of amyloid $\beta$ (A$\beta$) plaques and cerebral amyloid angiopathy (CAA) in United Kingdom and combined Swiss and Austrian patients receiving hGH injections or hDM grafts, respectively, suggesting that these patients may have been exposed not only to prions, but also A$\beta$ seeds.

To expand number of iCJD cases studied and address possible geographic bias, we have investigated the prevalence of A$\beta$ pathology in the following iCJD cases from US and other countries: (1) 14 neuropathologically confirmed US cases of iCJD (mean age±SD: 41±8 yrs range: 26-54 yrs), including 12 associated with hGH (hGH-CJD) and 2 associated with dura mater graft (hDM-CJD); (2) 11 non-US cases (41±19 yrs range: 14-71 yrs), exclusively hDM-CJD, including 3 from Canada, 6 from France and 2 from Australia; (3) 2 US subjects treated with hGH but iCJD free; (4) 65 control cases (43±8 yrs range: 23-57 yrs) including a) 35 cases of sporadic CJD (sCJD); b) 25 subjects with rapidly progressive non-CJD dementia and c) 5 autopsy cases of non-neurological disorders.

Preliminary analyses show that only 1 hGH-CJD and 1 hDM-CJD (44 and 26 yrs) of the 14 iCJD US cases (14%) showed abundant mature A$\beta$ plaques and CAA. Of the 3 non-US hDM-CJD cases so far examined, 2 showed A$\beta$mature plaques (age 49 yrs from Canada; 61 yrs from Australia) but only the latter case had CAA. No A$\beta$-positive iCJD case showed tau pathology. None of the 35 sCJD controls examined to date exhibited A$\beta$mature plaques and CAA.

Our results indicate that 1) significant and distinct A$\beta$ pathology with similar features may occur in iCJD cases from US, Canada and Australia (French cases are under study); 2) A$\beta$histopathological phenotype is tipically characterized by the co-occurrence of mature plaques and CAA; 3) this phenotype appears to be less frequent in the US than in the UK hGH-CJD (~8% vs ~50%); 4) it remains to be established whether the A$\beta$ pathology results from iatrogenic transmission, is facilitated by prion presence or represents an early pre-symptomatic AD.

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O-19: Prion acute synaptotoxicity at the CA1 region of the stratum radiatum

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Introduction: Although misfolding of normal prion protein (PrP$^C$) into abnormal conformers appears critical for disease transmission and thereby initiation of pathogenesis, the precise molecular species causing neurotoxicity in prion disease is unknown. Evidence supports that misfolded PrP conformers, probably as soluble oligomers, are likely to be a principal determinant of neurotoxicity but this remains unproven and the specific biophysical properties of the relevant toxic species are unknown. Hippocampal synaptotoxicity within the CA1 region of the stratum radiatum is reported as a relatively early feature of prion disease pathogenesis. Long-term potentiation (LTP) within the CA1 region is an important neurophysiological correlate of hippocampal-dependent declarative memory and LTP disruption has been exploited to model other synaptotoxic peptides, such as soluble oligomeric A$\beta$42.

Methods: Ex vivo prions/misfolded PrP were sourced from terminal prion (M1000 strain) disease mouse brains and chronically infected RK13 cell lines (expressing mouse PrP), including preparations following PrP immunodepletion and proteinase-K (PK) treatment to decrease total PrP species and specifically select for PK-resistant PrP species (PrPres), respectively. Synaptotoxicity in the form of disrupted LTP was assessed in an electrophysiological model employing Multichannel Electrode Arrays interrogating the Schäffer collateral pathway within the CA1 region, with hippocampal slices derived from C57BL/6 wild type and genetically matched PrP knockout mice following exposure to various ex vivo preparations.
**Results:** LTP from both 12-week and 11-month old wild type mice was significantly impaired by 24-27% after brief exposure to 0.5% (w/v) crude M1000 brain homogenates and infected 2% (w/v) RK13 lysates. The LTP impairment was associated with reduced post-tetanic potentiation suggesting likely concomitant presynaptic dysfunction. Specifically immuno-depleting 72% ± 9% of PrP species from M1000 brain homogenates significantly restored LTP by 70%, thus showing that the acute synaptotoxicity of ex vivo prion preparations is tightly linked to the presence of PrP species. Further, LTP remained significantly impaired by 34% following exposure to M1000 brain homogenate treated with 5 μg/ml PK (sufficient to completely degrade PrPc), demonstrating that PrPSc is intimately linked to acute prion synaptotoxicity. LTP disruption was confirmed to be PrPc independent, with PrPc knockout mice demonstrating significant impairment similar to wild type mice after exposure to crude M1000 brain homogenates. Studies of the pathophysiological basis to the synaptotoxicity employing biochemical analyses of treated hippocampal slices are ongoing.

**Conclusion:** We have developed a robust quantitative model of acute prion synaptotoxicity, demonstrating that PrPSc species appear directly responsible and can act independent of PrPc expression.

**O-20: Neuron and glial cell type-specific detection of PrPSc in prion-infected mouse brain by flow cytometry**

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Detailed analyses of PrPSc-positive cells in the central nervous system (CNS) are required to clarify pathological changes that occur in human and animals affected with prion diseases. Here, we report the establishment of a novel method for detection of PrPSc in neural cells by flow cytometry with the specific labeling of PrPSc using anti-PrP mAb 132. Cells were dissociated from brains with papain-based enzymes and fixed with paraformaldehyde. After treatment with GdnSCN, the cells were stained with mAb 132 for PrPSc and antibodies for neuronal and glial markers; MAP2 for neurons, GFAP for astrocytes and IBA1 for microglia. Cell nuclei were stained with 7-AAD and the cells were analyzed by flow cytometer. After gating of the cell bodies based on nuclear staining and forward and side scatter profiles, the double staining of PrPSc and cell markers clearly distinguished neurons, astrocytes and microglia positive for PrPSc from those negative for PrPSc. Next, we analyzed kinetics of PrPSc-positive cell populations in each cell type in the brains of mice infected with Chandler or Obihiro prion strains. Neurons, astrocytes and microglia positive for PrPSc became detected from 60 d post inoculation (dpi) and the PrPSc-positive cells increased in a time-dependent manner in the both strains. Interestingly, even though mice infected with the 2 strains succumb to the disease with nearly the same survival period, proportion of PrPSc-positive cells in each cell type increased earlier in Chandler strain-infected mice than in Obihiro strain-infected mice until 90 dpi, but reached to nearly the same levels from 120 to 145 dpi. Consistent with the finding in flow cytometric analysis, immunoblot analysis showed a rapid increase in the amount of PrPSc in mice infected with the Obihiro strain compared to those infected with the Chandler strain in the later stage of infection. These results suggest that propagation and cell-to-cell spread of the Chandler strain occur earlier than those of the Obihiro strain, but in the later stage of infection, the Obihiro strain propagates and spreads more efficiently than the Chandler strain. The flow cytometric detection of PrPSc revealed that the strain-specific characteristics of prion propagations in neurons and glial cells in the CNS. Additionally, this technique is applicable to the separation of PrPSc-positive neurons with fluorescence-activated cell sorting, and thus allows us to perform transcriptome analysis specific to...
prion-infected neurons for the identification of the neuronal cell types and the pathological changes in the neurons.

**O-21: Towards prophylactic treatments for carriers of pathogenic PrP mutations**

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Generating a treatment for genetic prion diseases is a special challenge since around each of these patients there is a group of family members carrying the dominant pathogenic mutation which are at imminent risk to develop this fatal disease at adult to old age. Therefore, efforts should be devoted to develop not only drugs for diagnosed patients, but also safe reagents for long term administration that can prevent/delay the onset of disease in subjects at risk. Our TgMHu2ME199K mice, which mimic for E200KCJD, are born healthy but subsequently present initial signs of neurodegenerative disease at 4-5 months old which aggravate to total paralysis at one year of age. Exacerbation of disease signs occurs concomitant with the increased accumulation of protease resistant PrP, which is undetectable in infant and very young mice. We have recently shown that Nano-PSO, a submicron formulation of Pomegranate Seed oil, can significantly delay the onset of disease when administered to 3 months old TgMHu2ME199K/ko mice by a neuroprotective mechanism which includes reduction in neuronal death and brain lipid oxidation. We now tested whether such clinical effect is increased when Nano-PSO administration to both TgMHu2ME199K/KO and TgMHu2ME199K/wt mice commenced at the day of birth, as compared to a similar Nano-Soya formulation. We found that both reagents had no deleterious effects on either pregnant or newborn mice, however only Nano-PSO delayed disease onset significantly in both Tg lines. While there was no significant difference in the total levels of PrPSc in treated and untreated brains as determined by immunoblots, pathological examination did show reduced levels of intracellular PrP as well as intracellular Periodic acid-Schiff (PAS) positive forms in Nano-PSO treated brains and retinas. Most important, there was no clinical advantage in initiating Nano-PSO administration to TgMHu2ME199K mice from day 1 as compared to 3 month of age. This suggests that anti prion treatment in carriers of pathogenic PrP mutations should commence at the time point in life in which the pathological aggregation starts. This time point in life may be different for each PrP mutation and for each of the other prion like diseases.

**O-22: Eradication of PrPSc by poly-L-arginine in cells infected with prions**

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Accumulation of infectious prion protein (PrPSc), the insoluble and protease-resistant pathogenic isoform of the cellular prion protein (PrP), is a hallmark in prion diseases. Previously, poly-L-lysine (PLK) was shown to inhibit prions in cell-based and animal models of prion disease. In present study, we report...
poly-L-arginine (PLR) as an anti-prion agent. PLR decreased PrPSc level in a dose- and time-dependent manner in multiple lines of cultured cells permanently infected with prions of different sources. Western blots of proteinase K-resistant PrPSc followed by densitometry analysis showed that PLR is more potent than PLK. The concentrations of PLR, at which PrPSc is completely eradicated, were not toxic for cells. PLR with different molecular weights showed prominent PrPSc inhibition, while PLR with higher molecular weights was more efficacious. The PrPC level was unaltered by treatment with PLR. PNGase-F experiment showed that PrPSc-associated C2 fragment disappeared but PrPC-associated C1 fragment remained the same as cells were treated with PLR. Thus, PrPSc inhibition by PLR does not occur through suppression of PrPC expression. In the PrPSc degradation assay, PLR did not deteriorate PrPSc in both low and neutral pHs, thus suggesting that degradation of PrPSc is not the action mechanism of PLR for its potency. PLR formed the complex with plasminogen more efficiently than PLK; this suggests that PLR action may occur through blocking the function of plasminogen, which assists in enhancing PrPSc propagation. PLR has high potency to eradicate PrPSc and highlights its potential role in prion therapeutics.

O-23: Pre-implantation exclusion of embryos at risk for prion diseases

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Genetic Creutzfeldt-Jakob disease (CJD) is a late-onset fatal degenerative brain disorder with autosomal dominant inheritance and near 100% penetrance. Jews of Libyan origin have an increased incidence of CJD due to a common founder mutation. We report preimplantation genetic diagnosis (PGD) by exclusion for CJD in embryos obtained from offspring of affected patients. PGD was applied in 8 CJD families for which the offspring did not wish to learn their carrier status, yet wanted to have non-carrier children. Following an IVF procedure, embryos that inherited the PRNP haplotype originating from the non-affected parent were identified and transferred. PGD by exclusion for CJD provides a non routine option for individuals who wish to avoid the transmission of the mutant gene without revealing their own carrier status.