TSE research uses a series of experimental TSE strains, many derived from the “SSBP/1” sheep brain pool, first passaged in sheep in 1945. We are reviewing the history of SSBP/1 in ruminants and rodents and TSE strains derived from it. SSBP/1 mouse passages yielded several strains including 22A, 22C and 22L. The 16th sheep passage was used to infect goats, where two “strains” were identified; one ("Drowsy") produced the first transmissions to rodents and gave mouse strains 79A, 79V, 139A, RML and hamster strain 263K. Diversity in experimental strains may arise from the presence of a mixture of strains in the original SSBP/1 pool. In addition sheep or goats used for passage of SSBP/1 may have been naturally infected, e.g., in the drowsy goat line. By analogy, endogenous infection of rodents with TSE-like agents is also possible, activated by experimental intervention. Selection of strains in mice also depends on PrP genotype. Changing PrP genotype sometimes leads to the selection of new strains, e.g., 22F when 22A was passaged in PrPaa mice; but sometimes original strain properties are retained e.g., cloned 22C passed in PrPbb mice. Experimental selection pressures are exercised by the choice of PrP genotype, route of infection, dilution and relative incubation period in the donor. They may also apply when TSE agents are replicated in vitro, e.g., using PMCA. Changing the environment for TSE replication may allow mutant strains to be selected, similarly to mechanisms proposed in the quasi-virus hypothesis. TSE passage histories illustrate how this may occur.
PPo2-3: Structural Comparisons of Full-length PrPSc Amyloid and Recombinant PrP(23–230) Amyloid Corroborate Differences in Biological Activity

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Key words: PrPSc amyloid, synthetic prions, protein structure, amyloid fibril

To date, there is conflicting experimental evidence regarding the ability of full-length PrPSc to form amyloid fibrils. In contrast, the equivalent, recombinant PrP [recPrP(23–230)] readily produces amyloid fibrils, some of which are infectious when inoculated into indicator mice. We discovered that PTA-precipitated PrPSc from infected brain forms amyloid fibrils in vitro. Electron microscopy revealed that the fibril diameter (5.7 ± 1.2 nm) and morphology were indistinguishable from those of PrP 27–30 fibrils (5.7 ± 1.1 nm). X-ray fiber diffraction confirmed that PrPSc amyloid contains a repeating unit of four beta-sheet strands in a cross-beta configuration, as seen with PrP 27–30. Surprisingly, the N-terminal domain of PrPSc does not contribute to the diameter and core structure of the amyloid fibril, which may contain a beta-helical fold. RecPrP(23–230) amyloid fibrils, which were generated by refolding under denaturing conditions, are structurally distinct from brain-derived PrPSc amyloid. Electron microscopy showed heterogeneous populations of fibrils with an average diameter of 7.1 ± 1.8 nm, while fiber diffraction indicated the presence of stacked beta-sheets that are typically seen in conventional amyloid. Immunohistochemistry and Thioflavin S-staining indicated the presence of full-length PrPSc in amyloid plaques from Syrian hamsters infected with Sc237 prions. Therefore, full-length PrPSc is able to form amyloid fibrils in vitro and in vivo, which are structurally distinct from fibrils formed by recPrP(23–230). Moreover, recPrP(23–230) amyloid is structurally heterogeneous, providing a possible explanation for its long incubation period and low apparent titer in bioassays, although alternative interpretations cannot be excluded.

PPo2-4: Neuropathological and Molecular Characterization of Rabbit in vitro Adapted BSE Upon Inoculation in Bovine PrP Transgenic Mouse Model

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Key words: BSE, PMCA, rabbit, in vivo, in vitro

Transmissible spongiform encephalopathies are group of diseases caused by prions. These infectious agents consist of misfolded conformers of cellular prion protein which are capable to transmit their properties to their cellular protein counterpart. One of the characteristics of prions is their ability to infect some species and not others. This phenomenon is known as transmission barrier. Compelling evidence indicates that the transmission barriers are closely related to differences in PrP amino acid sequences between the donor and recipients of infection, as well as the prion strain conformation, amongst other possible factors. Due to the absence of rabbit prions in Nature and unsuccessful results trying to infect experimentally rabbits using different prion species/strains, rabbit was considered a prion resistant species. We wanted to address this open question by using Protein Misfolding Cyclic Amplification (PMCA) technique that mimics in vitro some of the fundamental steps involved in prion replication in vivo albeit with accelerated kinetics. PMCA results suggested that rabbit should no longer be considered a prion resistant species. In order to evaluate the infectivity of the new rabbit adapted BSE prions, a bovine PrP transgenic mouse model was used. A detailed characterization of its pathogenesis suggests that the new rabbit prion strain is not only highly infectious but maintains the BSE prion strain properties. These studies are being very useful in evaluating new in vitro generated prion species as potential risks to humans and other animals.
PPO2-5: Biological Adaptation of Synthetic Prion Strains

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Key words: synthetic prions, prion strains, strain adaptation

The prion protein (PrP) is capable of folding into multiple self-replicating conformational strains associated with phenotypically distinct neurological disorders. The evolved structural properties of natural prion strains predict their phenotype and can breed true upon serial passage. Synthetic prion strains, formed by the in vitro misfolding of recombinant PrP, have not been exposed to extended periods of natural selection and have physicochemical properties that differ dramatically from natural strains. Here, we have repeatedly passaged mouse synthetic prion (MoSP) strains in mice and cultured cell lines, then monitored changes in their physicochemical properties over time. We show that MoSP strains gradually adopt properties associated with a well-characterized, natural prion strain, including shorter incubation times and lower conformational stabilities. Additionally, we observed a change in the molecular mass of the unglycosylated, protease-resistant fragments of the infectious prion conformer (rPrPSc), from 19 kDa to 21 kDa, although analysis of multiple MoSP strains indicate that this transition in not a universal feature of synthetic prion strain adaptation. Analysis of MoSP strain adaptation in cultured cells indicated that competition amongst subpopulations of co-existing strains directs the evolution of the bulk prion population. The external environment of the cell can dramatically influence the adaptation rate of MoSP strains. The presence of polyamidoamines in the culture media reverses the natural course of MoSP strain adaptation and favors conformational properties associated with synthetic strains. These findings demonstrate that prions are dynamic pathogens that leverage their conformational heterogeneity to acclimate to specific host environments.

PPO2-6: Molecular Characterisation of Seven Austrian BSE Isolates: Two L-Type Cases and a Special C-Type Case

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Key words: BSE, prion, diagnosis, strain type, subtype

Common bovine spongiform encephalopathy (C-type BSE) has been accounted for spread of the BSE-epidemic from UK to other countries. Rare aberrant BSE types H and L have been found in animals aging eight years and more. In Austria only seven cases of BSE have been detected. Two were from six year old animals, the others were derived from animals above age ten. Therefore, the potential presence of aberrant types was investigated. Similar methods as before published by Jacobs et al. (J Clin Microbiol 2007; 45:1821) were used; PrPres analysis was performed on western blots using group A, B and C antibodies as well as investigation of the susceptibility for digestion with proteinase K (PK). Five cases behaved as C-type, while two cases had typical properties of L-type BSE or BASE. The ages of these two animals were 11 and 12.5 yr. One of the C-type cases (age 12.5 yr) behaved peculiar in the way that its binding to antibodies 12B2 and P4 was relatively strong compared to that of core antibody L42. Finding all three different BSE types in cattle is most probable in cattle of 10 years and older. The fact that types L and H are also found in low incidence countries like Austria, Sweden, USA, Japan and Canada is indicative for natural presence of sporadic BSE in different forms. Safeguarding the food chain for BSE is only sensible when the old age animals are included in active monitoring (Funders: Dutch LNV ministry, Austrian AGES).

PPO2-7: Biochemical and Biophysical Characterization of Different CWD Isolates

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Key words: CWD, strains, FT-IR, AFM

Chronic wasting disease (CWD) is one of three naturally occurring forms of prion disease. The other two are Creutzfeldt-Jakob disease in humans and scrapie in sheep. CWD is contagious and affects captive as well as free ranging cervids. As long as there is no definite answer of whether CWD can breach the species barrier to humans precautionary measures especially for the protection of consumers need to be considered. In principle, different strains of CWD may be associated with different risks of transmission.
Prion strain interference can influence the emergence of a dominant strain from a mixture; however, the mechanisms underlying prion strain interference are poorly understood. Inoculation of a strain from a mixture; however, several different findings indicate that there exists more than one strain of CWD agent in cervids.

We have analysed a set of CWD isolates from white-tailed deer and could detect at least two biochemically different forms of disease-associated prion protein PrPTSE. Limited proteolysis with different concentrations of proteinase K and/or after exposure of PrPTSE to different pH-values or concentrations of Guanidinium hydrochloride resulted in distinct isolate-specific digestion patterns. Our CWD isolates were also examined in protein misfolding cyclic amplification studies. This showed different conversion activities for those isolates that had displayed significantly different sensitivities to limited proteolysis by PK in the biochemical experiments described above. We further applied Fourier transform infrared spectroscopy in combination with atomic force microscopy. This confirmed structural differences in the PrPTSE of at least two distinct CWD isolates.

The data presented here substantiate and expand previous reports on the existence of different CWD strains.

**PPo2-8: Co-Infecting Prion Strains Compete for a Limiting Cellular Resource**

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**Key words:** strain, interference

Prion strain interference can influence the emergence of a dominant strain from a mixture; however, the mechanisms underlying prion strain interference are poorly understood. Inoculation of the sciatic nerve with the drowsy (DY) strain of TME can completely block HY TME agent prior to superinfection with the sciatic nerve with the drowsy (DY) strain of the transmissible prion strain interference are poorly understood. Inoculation of a strain from a mixture; however, several different findings indicate that there exists more than one strain of CWD agent in cervids.

We have analysed a set of CWD isolates from white-tailed deer and could detect at least two biochemically different forms of disease-associated prion protein PrPTSE. Limited proteolysis with different concentrations of proteinase K and/or after exposure of PrPTSE to different pH-values or concentrations of Guanidinium hydrochloride resulted in distinct isolate-specific digestion patterns. Our CWD isolates were also examined in protein misfolding cyclic amplification studies. This showed different conversion activities for those isolates that had displayed significantly different sensitivities to limited proteolysis by PK in the biochemical experiments described above. We further applied Fourier transform infrared spectroscopy in combination with atomic force microscopy. This confirmed structural differences in the PrPTSE of at least two distinct CWD isolates.

The data presented here substantiate and expand previous reports on the existence of different CWD strains.

**PPo2-9: Significance of Murine Scrapie Strains: ME7 and 22a**

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**Key words:** strain concept, strain diversity, strain stability, host-donor interaction, scrapie, pathogenesis

The idea that scrapie might exist in more than one strain arose originally from the recognition of different disease phenotypes in mice inoculated with natural sheep scrapie isolates. We showed that murine ME7 scrapie loses its distinct phenotype in mice after previous passage in sheep questioning the significance of the mouse bioassay as the gold standard method for strain characterisation and the stability of cloned strains. We have now analysed cloned murine 22a strain to determine (i) whether 22a produces a unique disease phenotype upon passage in sheep and (ii) whether its characteristic phenotype is recovered when re-isolated in mice. The comparison of these data with those from the ME7 experiment will help us to gain understanding about strains. Sheep homozygous for the VRQ or ARQ PRNP allele were orally (n = 22) or orally and parenterally (n = 18) challenged with ME7 or 22a. Sheep were monitored for signs of scrapie and killed at clinical end point. Tissue samples were labelled for disease-associated PrP, and selected positive brain inocula from “sheep-ME7 and sheep-22a” were inoculated into C57BL or VM mice, respectively.

Our results (i) confirm ME7 as being more unstable than 22a, (ii) demonstrate the generation of new “strains” according to current methods for strain characterisation and discrimination and (iii) highlight a major detrimental effect of the ovine VRQ allele for murine strain stability. These findings suggest that strains can result from an interaction between the agent and the genotype of the host, thus having profound implications for the prion strain concept.
PPo2-10: Conformational Stability of Prion Strains

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Key words: strains, conformational stability

Prion strains are stable, distinct phenotypes of disease characterized by differences in incubation period and neuropathology. Strain-specific biochemical properties of PrPSc have been observed. One of these biochemical properties is conformational stability, the ability of PrPSc to resist denaturation. We determined the incubation period and conformational stability of PrPSc of hamsters infected with either the HY TME, 263K, HaCWD, 22AH, 22CH, 139H, DY TME or ME7H strains. The incubation periods of these strains ranged from 61 to 263 days. The conformational stability of PrPSc was determined using guanidine hydrochloride (Gdn-HCl) or sodium dodecyl sulfate (SDS). Briefly, brain homogenate from each of the strains was incubated in 0–2% SDS (final w/v concentration in DPBS) or 0–2 M Gdn-HCl (final concentration in DPBS) at 70°C and proteinase K resistant PrPSc was quantified using a 96-well dot blot. For each concentration of denaturing agent, the level of PrPSc was standardized and the relationship between the abundance of PrPSc and the concentration of the denaturing agent was determined. For both denaturing agents, the shorter incubation period strains had more stable PrPSc compared to long incubation period strains. The inverse relationship between incubation period and stability is consistent with earlier reports examining hamster strains. This data is inconsistent with the hypothesis that decreased conformational stability results in enhanced PrPSc fragmentation and a shorter incubation period.

PPo2-11: Histopathological Analysis of TgOvPrP4 Mouse Brains Infected with 12 Isolates of French Atypical Scrapie

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Key words: Nor98, scrapie, TgovPrP4, strain typing

Introduction. Atypical scrapie or Nor98 is a transmissible spongiform encephalopathy clearly distinguishable from classical scrapie and from ovine BSE, notably regarding the biochemical features of the protease-resistant prion protein PrPres and the genetic factors involved in susceptibility to the disease. Transmission studies in transgenic mouse models overexpressing the ovine PrPVRQ protein have previously shown transmissibility of the disease from such isolates, but also revealed the uniform features and similarities of cases previously described in Norway, then in France and Germany. More recently our group described successful transmission of such isolates to the transgenic mouse model (TgOvPrP4) overexpressing in the brain the ovine PrPARQ protein.1 Here, in order to complete these biochemical analyses we characterised the Nor98 strain on the basis of histopathological features detected in the brain of TgOvPrP4 transgenic mice infected with French cases of atypical scrapie.

Results. The lesion profile as well as PrPd topographical distribution showed complete similarity among the 12 isolates transmitted and clear differences compared to histopathological data observed with the classical scrapie or ovine BSE sources.

Materials and Methods. 12 French atypical scrapie, one classical scrapie and one ovine BSE isolates, were used in transmission studies to the TgOvPrP4 mouse. Lesion profiles were analysed as well as typographical and topographical expression of the pathological form of PrP(PrPd) using immunohistochemistry (IHC).

Conclusion. The uniform histopathological features found in the brains of the TgOvPrP4 mouse infected with the 12 different atypical scrapie sources comforts the uniform biochemical features reported previously.

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PPo2-12: Limiting PMCA Amplification of Prion Strains

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Key words: prion, strain, HY, DY, 22AH, 22CH, 139H, ME7H, TME, HaCWD, PMCA, hamster

Prion strains are defined by a characteristic set of features, such as incubation period and neuropathology, that breed true upon experimental passage. In this study we investigated the relationship between the incubation period and the in vitro amplification rate of PrPSc. To determine if differences exist in the rate of PrPSc replication between strains, PMCA was performed on eight hamster-adapted prion strains. Brain homogenates were prepared from animals at the clinical stage of disease following inoculation of each strain, including an uninfected (mock) negative control, and serial 10-fold serial dilutions of these homogenates were subjected to one round of PMCA. One round of PMCA amplified PrPSc to detectable levels by western blot analysis in PMCA reactions that were initially seeded with 500 to 0.05 g-eq from HY TME infected animals. However, PMCA on brain homogenate from DY TME infected animals amplified PrPSc to detectable levels in reactions that were initially seeded with 500 to 5 g eq, but was not detected in PMCA reactions seeded with lower concentrations. This was the general trend, as the short incubation period strains HY TME, 263K and HaCWD replicated PrPSc at much higher dilutions compared to the long incubation period strains 22AH, 22CH, 139H, DY TME and ME7H, suggesting
that differences exist in the kinetics of PrPSc replication between strains. This study shows that PMCA can effectively amplify many different hamster prion strains, their amplification rate is an inherited property of each strain and that the rate of PrPSc amplification correlates with incubation period.

PPo2-13: Distribution of Pathological Form of Prion Protein in Brainstem Samples from Cases of Classical and Atypical BSE

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Key words: bovine spongiform encephalopathy, BSE, prion protein, PrP distribution, atypical BSE

In recent years two new types of BSE were discovered in several countries. L-type and H-type atypical BSE was found only in older cattle and majority of cases showed no clinical signs of the disease. While in atypical scrapie in many cases cerebellum harbours higher amount of prion protein resistant to proteolysis (PrPres) when compared to obex, no such data is available for atypical BSE. In this study we evaluated the distribution of prion protein resistant to proteolysis (PrPres) in brainstem samples of cattle affected with classical and atypical BSE using western blot technique. Surprisingly in both classical and atypical BSE stronger signal in comparison to obex was observed in cranial parts of the brainstem samples. While in classical and H-type BSE cases significant drop in PrPres amount was noticed in cerebellum in comparison to obex, L-type BSE was characterized by similar signal strength in both regions. Uniform distribution of PrPres especially in the cranial to obex part of brainstem shows that when autolysed sample is tested for BSE it is possible that both classical and atypical BSE might be detected even when the target site is hard to identify or is missing.

PPo2-14: L-type and Epizootic BSEs Playing Cat and Mouse

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Key words: prion, BSE, atypical BSE, strain, shift

The active, large-scale testing of livestock nervous tissues for the presence of protease-resistant prion protein (PrPres) has led to the recognition of two uncommon PrPres molecular signatures, termed H-type and L-type BSE. Their experimental transmission to various laboratory models unambiguously demonstrated the infectious nature of such cases and the existence of distinct prion strains in cattle. However L-type prions acquired molecular and neuropathologic phenotypic traits undistinguishable from BSE or BSE-related agents upon transmission to transgenic mice expressing the VRQ allele of ovine PrP (tg338 line). A similar emergence of a BSE-like phenotype was found by others in wild-type mice, leading to the hypothesis that L-type may be at the origin of epizootic BSE.

We have pursued the serial transmission of (biologically cloned or not) BSE and L-type prions in tg338 mice over six passages and studied their phenotype in details. Although their pathological and neuropathological traits still appeared similar, the ability of the two agents to replicate in the spleens strongly differed, both in terms of PrPres accumulation and infectious titre. When spleens and brain tissues from tg338 mice infected with L-type and BSE prions were transmitted back to bovine PrP transgenic mice, the bovine, specific phenotype of each agent was fully restored, further suggesting that the tg338 passaged agents, although producing a similar signature in the brain were actually different.

Beyond providing another illustration of the so-called convergence phenomenon, these data provide evidence that the latter can be tissue-specific and further lend support for additional strain typing criteria in non-neuronal tissues to distinguish between TSE agents.

PPo2-15: Molecular Prion Protein Typing in Atypical/Nor98 and Classical Scrapie Affected Transgenic Mice

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Key words: scrapie, prion protein, atypical scrapie, transgenic mice

A particular feature of atypical/Nor98 scrapie is a western blot pattern of the proteasome K (PK) resistant prion protein (PrPres) fragments clearly different from other prion diseases in small ruminants. This pattern consists of a predominant unglycosylated PrPSc peptide that migrates below 12 kDa and multiple bands in the range of 12–30 kDa, which have been proposed to represent un-, mono- and diglycosylated moieties of at least one additional fragment. The aim of this study was to investigate by western blot whether these fragments are generated by PK cleavage in-vitro or rather by proteolysis in vivo and to identify any qualitative differences in the total of PrP (PrPSc+c) between atypical scrapie, classical scrapie and healthy animals. We analyzed brain tissues of non-inoculated healthy and terminal stage diseased Tg338 mice that had been inoculated with isolates of atypical/Nor98 (n = 4) as well as classical scrapie (n = 1). After PK
with ARR/ARR sheep showing a lower relative ratio and a lower glycotype compared to the other genotypes. An opposite, but lower effect was observed in ARQ/AHQ sheep. These results suggest that the proteinase K cleavage site of BSE PrPres can be altered by PrP genotype. Notwithstanding, ISSDWB allowed to discriminate all scrapie samples from BSE. Further analyses are needed to evaluate the effects of host genotype and route of inoculation on sheep-adapted BSE.

PPo2-17: Atypical H-type BSE Infection in Bovine-PrP Transgenic Mice Let to the Emergence of Classical BSE Strain Features

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Key words: atypical BSE, PrPres, prion strain, prion transmission

Until identification of atypical cases of Bovine Spongiform Encephalopathy (BSE) in several countries it was assumed that BSE in cattle consisted of only a unique and biologically homogeneous strain type that caused BSE epidemic in Europe. Currently, besides the classical BSE strain associated to most described cases, atypical BSE cases are identified as H- or L-type based on the differences in the western blot profiles of abnormal protease-resistant prion protein (PrPres) according to the apparent molecular mass of its unglycosilated band. In the present study, we characterized five atypical BSE-H isolates by analyzing their molecular and neuropathological properties after transmission in transgenic mice expressing homologous bovine prion protein (PrP). The results showed that most of the inoculated animals conserved the atypical BSE-H strain features. However, a number of animals inoculated with two of these isolates showed prion strain features resembling those of classical BSE in this mouse model. On each case, the strain characteristics were preserved after subsequent passage in the same mice. These data suggest that atypical BSE-H prions, can acquire epidemic BSE-like properties during propagation in a homologous bovine PrP context. Beside a new view on BSE strains diversification, our observations support the hypothesis that atypical BSE-H, which could be a sporadic form of prion disease in cattle, may be at the origin of the foodborne BSE epizooty.
PPo2-18: PrP Genetics, Molecular Characterization and Biological Typing of Natural Goat Scrapie Isolates from Greece

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Key words: goat scrapie, PRNP polymorphisms, discriminatory WB, strain typing

Greece has the second highest scrapie prevalence in EU but limited data are available on the characterization of scrapie strains. Our aims were to investigate the PRNP gene variability of Greek goats, to discriminate goat scrapie from BSE and to characterize Greek scrapie isolates by bioassay.

The PRNP gene CDS of 32 scrapie positive and 78 negative goats was analyzed. Fifteen goat isolates were submitted to discriminatory WB; ten of them were also analysed by bioassay in Tg338 mice and bank voles, together with two sheep isolates from a mixed herd.

Seventeen PRNP polymorphisms and an octapeptide deletion polymorphism were observed in goats. 173 S/N, 239 S/F substitutions and the octapeptide deletion (codons 70–77) are described for the first time. Variants of seven polymorphisms were observed only in the control group. Polymorphisms known to be associated to scrapie protection (143R, 146S and 222K) were observed only in scrapie negative goats, suggesting also in Greece their potential protective role. By discriminatory western-blot, all isolates showed the typical molecular pattern of classical scrapie and were clearly distinguishable from sheep BSE. No BSE-like cases were detected. Preliminary results of bioassays indicate the circulation of more than one scrapie strain in goats from Greece. Indeed, goats from six herds were transmitted with short incubation time to bank voles (<200 dpi), but not to Tg338 mice (>350 dpi); conversely, both sheep and goat isolates from the same mixed herd gave the opposite pattern of transmission (around 200 dpi in Tg338 mice, >300 dpi in bank voles).

PPo2-19: Translational Control of RML and 22L Prions by MAP Kinase Signaling in GT1-1 Cells

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Key words: prion, MAP kinase, ERK, MEK, RML, 22L

We have previously observed that exposure to brain-derived neurotrophic factor and depolarization with high [KCl] of RML prion strain-infected GT1-1 cells increase formation of prions. Both treatments activate the MAP-kinase ERK pathway. Inhibition of the ERK pathway, using specific MEK-inhibitors, effectively cleared GT1-1 cells from the misfolded prion protein (PrPSc), but had no effect on the normal cellular PrPC. Inhibition of the p38 and JNK pathways had the opposing effect and instead caused increased formation of PrPSc. This indicates that conversion of the cellular prion protein, PrPC, to its pathogenic isoform, PrPSc, can be regulated by physiological stimuli acting on specific signal transduction pathways.

We have now compared the activation of MAP kinase signaling in RML strain-infected cells with 22L-infected cells, which express higher levels of PrPSc than the former. The 22L strain-infected cells displayed stronger activation of the MEK/ERK pathway as revealed by phospho-ERK levels. The RNA-expression profiles of MAP kinase signaling related transcripts did not differ between uninfected GT1-1 cells and cells infected with the RML and 22L strains. On the other hand, the cytoplasmic S6 ribosomal protein was activated as revealed by increased phosphorylation of both the S235/36 and S240/44 sites when the cells were exposed to high [KCl] leading to increased prion formation. The present results strengthen the hypothesis that formation of PrPSc may be regulated at a translational rather than at a transcriptional level, but that the level of activation of these signaling pathways may vary between different prion strains.

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Infectious prions exist in a variety of strains, which are thought to differ in the conformation or oligomerization state of PrP\(^{Sc}\). As a consequence, strains may have a different population of aggregates which would contribute to their unique toxic, infectious and replicative characteristics. In this work we describe a method to separate PrP\(^{Sc}\) aggregates of different sizes under native conditions based on ultracentrifugation in density gradients. Our results show that the size-distribution of PrP\(^{Sc}\) aggregates associated with two biologically similar hamster strains 263K and HY was very similar. In these strains, PrP\(^{Sc}\) aggregates appeared in two main peaks, one composed of large aggregates that were retrieved in the pellet fraction and a broad peak with gaussian distribution in the middle of the gradient. The size distribution of the DY prion strain, on the other hand, exhibited substantial differences from 263K and HY. DY PrP\(^{Sc}\) showed a new peak composed of smaller and PK-sensitive oligomers and the complete absence of large PrP\(^{Sc}\) aggregates in the pellet. These experiments were done by using both partially purified PrP\(^{Sc}\) and complete brain homogenates and the results were similar with both materials, indicating that the preparation of the sample was not responsible for the size-distribution results. Citotoxicity of different PrP\(^{Sc}\) aggregates was assessed in culture cells and the results show that mid-range PrP\(^{Sc}\) aggregates and smaller oligomers were more toxic that larger fibrillar structures. Our results may contribute to understand the mechanisms of strain variation and the role of PrP\(^{Sc}\) aggregates in prion-induced neurodegeneration.

**PPo2-20: Evaluation of PrP\(^{Sc}\) Aggregation in Different Prion Strains**

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Conformational Stability Assay

Denaturation curves showed strain-specific conformational stabilities, with [GdnHCl]\(^{1/2}\) values ranging from 1.96 to 2.31 M for classical scrapie, from 2.06 to 2.88 M for CH1641 and CH1641-like and from 1.26 to 1.43 M for Nor98. BSE samples showed the highest conformational stability compared to all other samples, with [GdnHCl]\(^{1/2}\) values >3, 5 M. Our preliminary results suggest that CSSA reveals strain-specific PrP\(^{Sc}\) conformational stabilities of ovine prion isolates. The higher conformational stability of sheep BSE compared to all other known TSEs of sheep could be exploited for a more reliable and unequivocal scrapie/BSE discrimination.

**PPo2-21: Discrimination of Ovine BSE from Classical Scrapie, CH1641-like and Nor98 Isolates by a Novel Conformational Stability Assay**

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Key words: strain, scrapie, CH1641_like, ovine BSE, conformational stability

TSEs of small ruminants include classical and atypical (Nor98) scrapie, which can be discriminated from experimental ovine BSE by PrPres molecular typing. However, some natural sheep isolates have been described in sheep showing molecular similarities with BSE, as determined by the PK cleavage site of PrP\(^{Sc}\). These were named CH1641-like due to molecular similarities with the experimental CH1641 strain.

Aim of this study was to analyse the conformational stability of PrP\(^{Sc}\) from sheep TSE isolates by a new conformational stability and solubility assay (CSSA) that we have recently developed. CSSA employs denaturation with increasing concentrations of GdnHCl and differential centrifugation and allows to determine the [GdnHCl]\(^{1/2}\) value of PrP\(^{Sc}\) aggregates without PK digestion.

CNS samples from sheep with natural scrapie (n = 13), experimental CH1641 (n = 1) and experimental BSE (n = 3) were analysed. The 13 natural scrapie isolates included classical scrapie (n = 5), Nor98 (n = 5) and CH1641-like (n = 3) cases. Denaturation curves showed strain-specific conformational stabilities, with [GdnHCl]\(^{1/2}\) values ranging from 1.96 to 2.31 M for classical scrapie, from 2.06 to 2.88 M for CH1641 and CH1641-like and from 1.26 to 1.43 M for Nor98. BSE samples showed the highest conformational stability compared to all other samples, with [GdnHCl]\(^{1/2}\) values >3, 5 M. Our preliminary results suggest that CSSA reveals strain-specific PrP\(^{Sc}\) conformational stabilities of ovine prion isolates. The higher conformational stability of sheep BSE compared to all other known TSEs of sheep could be exploited for a more reliable and unequivocal scrapie/BSE discrimination.

**PPo2-22: CWD Strain Emergence in Orally Inoculated White-tailed Deer (Odocoileus virginianus) with Different PRNP Genotypes**

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Key words: CWD, strains, emergence

Chronic wasting disease (CWD) is a prion disease affecting captive and free-ranging cervids in North America. We have previously demonstrated that specific Prnp polymorphisms are linked to susceptibility/resistance to CWD infection in free-ranging white-tailed deer populations. The “wild-type” alleles (with glutamine at aa 95 and a Glycine at aa 96) were over-represented in the infected deer while the polymorphisms at aa 95 (Q95H) and 96 (G96S) were under-represented in the CWD-positive animals. Experimental oral infection of white-tailed deer with known Prnp genotypes (with inocula from CWD-positive wt/ wt deer) confirmed this link between Prnp primary sequence and incubation period. All orally infected animals became clinically positive for CWD. The wt/wt had the shortest incubation period (693 dpi) and the Q95H/G96S the longest (1596 dpi). Brain homogenates prepared from clinically affected deer of each genotype were treated with proteinase K and resolved by western blot; differences in the glycosylation pattern and PK resistance were observed and are suggestive of different PrP\(^{Sc}\) isoforms.
Feline spongiform encephalopathies (FSE) have been reported in the last two decades in domestic as well as in captive wild cats. Here we report a FSE case in a nine year old female cheetah, which was born in The Netherlands and exported to Germany. This case was confirmed in 2007 on the basis of CNS samples. We have looked also at the PrPSc depositions in the peripheral nervous system as well as in muscular and parenchymal tissues by immunoblotting and IHC techniques showing an extensive PrPSc dissemination with a BSE-like pattern. Moreover, using the Scrapie Cell Assay (SCA) to measure infectivity, we observed, at low frequency, positive assay results in control groups. To gain insights into prion propagation to the cell surface or endocytic vesicles. Until recently little was known about the capacity of cytosolic amyloidogenic proteins to propagate as infectious protein assemblies. To gain insights into prion-like replication and spreading mechanisms of cytosolic prions, we have studied the aggregation propensities of the PrPSc deposits in the peripheral nervous system as well as in muscular and parenchymal tissues by immunoblotting and IHC techniques showing an extensive PrPSc dissemination with a BSE-like pattern. Moreover, we have used for the first time the PMCA method to amplify FSE PrPSc using bovine PrPSc from tgbv mouse brains as PrPSc substrate. In conclusion, our findings provide further evidence, for a possible link between FSE and BSE. The similarities of both strains suggest that BSE and FSE in different feline species may originate from the same infectious agent.
attachment is not a general requirement for prion transmission in mammalian cells.

**PPo2-26: Transmission of Classical and Atypical (L-type) Bovine Spongiform Encephalopathy (BSE) Prions to Cynomolgus macaques**

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Key words: L-type BSE, cBSE, cynomolgus macaques, transmission

BSE prion derived from classical BSE (cBSE) or L-type BSE was characterized by inoculation into the brain of *cynomolgus macaques*. The neurologic manifestation was developed in all *cynomolgus macaques* at 27–43 months after intracerebral inoculation of brain homogenate from cBSE-affected cattle (BSE JP/6). Second transmission of cBSE from macaque to macaque shortened incubation period to 13–18 months. cBSE-affected macaques showed the similar clinical signs including hyperekplexia, tremor and paralysis in both primary and second transmission. Two macaques were intracerebrally inoculated brain homogenate from the L-type BSE-affected cattle (BSE JP/24). The incubation periods were 19–20 months in primary transmission. The clinical course of the L-type BSE-affected macaques differed from that in cBSE-affected macaques in the points of severe myoclonus without hyperekplexia. The glycoform profile of PrPSc detected in macaque CNS was consistent with original pattern of either cBSE or L-typeBSE PrPSc, respectively. Although severe spongiform change in the brain was remarkable in all BSE-affected macaques, severe spongiform spread widely in cerebral cortex in L-type BSE-affected macaques. Heavy accumulation of PrPSc surrounded by vacuola formed florid plaques in cerebral cortex of cBSE-affected macaques. Deposit of PrPSc in L-type BSE-affected macaque was weak and diffuse synaptic pattern in cerebrum, but large PrPSc plaques were evident at cerebellum. MRI analysis, T2, T1, DW and flair sequences, at the time of autopsy revealed that brain atrophy and dilatation of cerebral ventricles were significantly severe in L-type BSE-affected macaques. These results suggest that L-type BSE is more virulent strain to primates comparing to cBSE.

**PPo2-27: Generation of a Novel form of Human PrPSc by Inter-species Transmission of Cervid Prions**

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Prion diseases are infectious neurodegenerative disorders affecting humans and animals that result from the conversion of normal prion protein (PrPC) into the misfolded and infectious prion (PrPSc). Chronic wasting disease (CWD) of cervids is a prion disorder of increasing prevalence within the United States that affects a large population of wild and captive deer and elk. CWD is highly contagious and its origin, mechanism of transmission and exact prevalence are currently unclear. The risk of transmission of CWD to humans is unknown. Defining that risk is of utmost importance, considering that people have been infected by animal prions, resulting in new fatal diseases. To study the possibility that human PrPSc can be converted into the infectious form by CWD PrPSc we performed experiments using the Protein Misfolding Cyclic Amplification (PMCA) technique, which mimics in vitro the process of prion replication. Our results show that cervid PrPSc can induce the pathological conversion of human PrPSc, but only after the CWD prion strain has been stabilized by successive passages in vitro or in vivo. Interestingly, this newly generated human PrPSc exhibits a distinct biochemical pattern that differs from any of the currently known forms of human PrPSc, indicating that it corresponds to a novel human prion strain. Our findings suggest that CWD prions have the capability to infect humans, and that this ability depends on CWD strain adaptation, implying that the risk for human health progressively increases with the spread of CWD among cervids.
Epidemiological connection to human disease has been revealed to date, scrapie is considered non-pathogenic for humans, at least under natural conditions. However, since it has been shown that sheep could be experimentally infected with BSE, possibility has been raised that BSE could have been accidentally introduced in this species. This has prompted in 2002 a surveillance plan for scrapie in small ruminants by the European Union in all member states.

In 2005, the first natural BSE case was identified in a French goat and four years later, a Scottish goat, from a retrospective study, showed bioassay results indistinguishable from BSE (STEG, 2009).

Although natural classical caprine scrapie is considered rare, it has been reported in many countries. Regarding atypical scrapie in goats data is very limited, representing 1–2% of scrapie positive cases in EU-27. They have been reported in France, Italy, Spain and Portugal (EC, 2008).

Between 2003 and 2009, a total of 415,340 small ruminants were screened and atypical scrapie was identified in 365 Portuguese sheep and 4 goats.

In the present study, we aimed to describe the phenotype features as well as Prnp sequence of these atypical caprine scrapie cases, comparing to previous reported data and thus contributing to understand this form of scrapie in this species.

PPo2-28: Mammalian Prions Generated from Bacterially Expressed Prion Protein in the Absence of any Mammalian Cofactors

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Key words: prions, protein misfolding, amyloid, protein folding, neurological diseases

Transmissible spongiform encephalopathies (TSEs) are a group of neurodegenerative diseases that are associated with the conformational conversion of a normal prion protein, PrP(C), to a misfolded aggregated form, PrP(Sc). The protein-only hypothesis asserts that PrP(Sc) itself represents the infectious TSE agent. Although this model is supported by rapidly growing experimental data, unequivocal proof has been elusive. The protein misfolding cyclic amplification reactions have been recently shown to propagate prions using brain-derived or recombinant prion protein, but only in the presence of additional cofactors such as nucleic acids and lipids. Here, using a protein misfolding cyclic amplification variation, we show that prions causing transmissible spongiform encephalopathy in wild-type hamsters can be generated solely from highly purified, bacterially expressed recombinant hamster prion protein without any mammalian or synthetic cofactors (other than buffer salts and detergent). These findings provide strong support for the protein-only hypothesis of TSE diseases, as well as argue that cofactors such as nucleic acids, other polyanions or lipids are non-obligatory for prion protein conversion to the infectious form.

PPo2-29: Atypical Scrapie in Goats-Pathological and Epidemiological Characterization and Relevance

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Key words: caprines, atypical scrapie, pathology, epidemiology, prnp genotype

Scrapie affects sheep and goats, being the most common form of transmissible spongiform encephalopathies, which include Creutzfeldt-Jakob Disease (CJD) in humans and Bovine Spongiform Encephalopathy (BSE), recognized for over 250 years in several European countries. As no obvious clinical or epidemiological connection to human disease has been revealed to date, scrapie is considered non-pathogenic for humans, at least under natural conditions. However, since it has been shown that sheep could be experimentally infected with BSE, possibility has been raised that BSE could have been accidentally introduced in this species. This has prompted in 2002 a surveillance plan for scrapie in small ruminants by the European Union in all member states.

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