

# Oral Presentations

## International Prion Congress Prion 2012

May 9–12, 2012, Amsterdam, The Netherlands

PRION 2012 is the leading international conference on TSEs held annually. This series of meetings was initiated in 2004 by Neuroprion as an European Network of Excellence, and since 2010 has been fostered by Neuroprion as an international association based in France (association@neuroprion.org). The science in this Prion 2012 abstract book represents the core business of the congress, which is defining the behavior and true nature of prions and their associated processes in transmissible spongiform encephalopathies. This is indispensable information to allow for targeted diagnosis, prevention and treatment of this family of infectious diseases. As a bystander result this knowledge will support further understanding of other protein folding diseases. Therefore, there has been created room for knowledge dissemination concerning neurological diseases like Alzheimer and Parkinson disease and amyotrophic lateral sclerosis (ALS) which will serve to understand the destroying mechanisms of pathological protein aggregation processes.

### OR-01: The challenge of structural understanding of prion protein conversion and prion propagation

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While great progress has been recently made toward proving the ‘protein-only’ nature of the infectious prion agent, the mechanism of the  $\text{PrP}^{\text{C}} > \text{PrP}^{\text{Sc}}$  conversion and, especially, the structural aspects of this process remain poorly understood. The lack of information in this regard represents a critical gap in prion research, hindering efforts to understand the molecular basis of TSE diseases as well as development of effective strategies for pharmacological intervention. Here we will review recent progress in this area of research, with a special emphasis on the structure of the infectious  $\text{PrP}^{\text{Sc}}$  conformer and the structural basis of prion strains. We will also describe novel experimental data that shed new light on the structural basis of prion propagation as well as the nature of critical nucleation elements in prion protein conversion.

### OR-02: Intrinsic structural and functional properties of experimental TSE strains

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The phenotypic properties of TSE strains can be characterized by differences in incubation period in panels of inbred mice, the amount and distribution of pathology, and the biochemical properties of abnormal forms of PrP. They also exhibit differences in thermostability, intrinsic to TSE agent structures.<sup>1</sup> Over many years several sources of TSE infectivity have been used to establish serial passage lines in mouse strains differing in PrP genotype

and in other rodents. Analysis of passage histories of individual sources of TSEs reveals many different strain phenotypes. For example at least nine phenotypes can now be identified from the SSBP/1 source and 15 from the Drowsy Goat source. In these cases the new phenotypes are primarily associated with changes in incubation period in one or two PrP genotypes of mouse, or different phenotypic properties in rats or hamsters. Passage into a new PrP genotype can result in zero, one, two or more identifiable changes in TSE agent phenotype, changes which occur over several passes in the new PrP genotype. A limited number of these changes may be attributable to pre-existing mixtures in the original TSE source. However the large number of novel phenotypes, some emerging after complex passage histories, and sometimes including passage at high dilution, suggests that many are attributable to high rates of mutational change to the TSE agent. Indeed TSE agents appear to have structures that can alter substantially and rapidly when their environment changes and there is an evolutionary path for them to do so. Accordingly several questions must be addressed. These include: (1) how are the structures of such a large number of TSE strains maintained; (2) what structural properties maintain thermostability diversity; (3) are the hypotheses of agent structure compatible with thermodynamic considerations; and (4) what is the molecular mechanism of mutation?

PrP binds specifically to some nucleic acids, notably the HIV genome where it negatively affects its translation.<sup>2</sup> PrP therefore shows functional properties toward nucleic acids which are compatible with the binding and protection of TSE-specific nucleic acid genomes, consistent with the virino hypothesis. This hypothesis proposes a structure of the agent comprising a TSE-specific nucleic acid genome protected by PrP. More specifically, the interaction between PrP and TSE-specific nucleic acids may be directly affected by mutational change to either macromolecule, hence controlling TSE agent replication and expression of TSE agent-host specific phenotypes.

By contrast the prion hypothesis proposes that PrP adopts abnormal conformations that cause the protein to become

infectious and encode properties specific to TSE agents and not encoded by the host. However the diversity of phenotypic properties of TSE agent strains provides a substantial challenge to this hypothesis. It requires a large number of abnormal conformations to be viable and sufficiently thermodynamically stable to differentially retain the genetic information required to specify each TSE strain phenotype. Indeed each additional phenotype further extends the challenge to an abnormal conformations hypothesis but remains entirely consistent with a TSE agent-specific nucleic acid genome.

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### OR-03: A compact, four-stranded $\beta$ -sheet core is found in prions from all natural prion isolates and synthetic prion strains analyzed

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To date, only limited information is available about the structure of the infectious prion protein, PrP<sup>Sc</sup>, and its proteolytically truncated homolog, PrP 27–30. In the absence of an experimental structure, molecular modeling has been used extensively to predict the structure of PrP<sup>Sc</sup>, but different modeling approaches have produced a large number of alternative models. Moreover, little consensus exists on the interpretation of the available data and the underlying structure of PrP<sup>Sc</sup>.

Recently, X-ray fiber diffraction indicated that rodent-adapted sheep prions (RML and Sc237 strains) contain a compact, four-stranded  $\beta$ -sheet core, possibly in a  $\beta$ -solenoid or  $\beta$ -helical configuration. A synthetic prion strain (MoSP1) propagated in transgenic mice displayed the same structural architecture. In all three cases, the PrP 27–30 fibrils exhibited a repeating unit of 19.2 Å per molecule, representing four  $\beta$ -sheet strands in a cross- $\beta$  arrangement. Negative-stain electron microscopy revealed the average fibril diameter to range from 48 to 57 Å, confirming the compact nature of the  $\beta$ -sheet core.

Here, we extended our analyses to eight natural prion isolates, six synthetic prion strains, and one inherited human prion disease model. In detail, we analyzed rodent-adapted prions from bovine spongiform encephalopathy (301V), transmissible mink encephalopathy (Drowsy and Hyper), chronic wasting disease (CWD), sheep scrapie (SSBP1, 139H, RML, and Sc237), and synthetic prion strains (MoSP1, MoSP2, MoSP5, MoSP6, MoSP7, and

SSLOW) that were generated by different investigators. Prions isolated from the brains of uninoculated transgenic mice expressing mutant PrP(P101L), which is analogous to the mutation causing Gerstmann-Sträussler-Scheinker syndrome (GSS) in humans, were also evaluated. These mice develop a spontaneous prion disease in ~200 d that mimics GSS. The diffraction patterns from all prion strains exhibited the same repeating unit of 19.2 Å per molecule, represented by a series of meridional diffraction signals at 4.8, 6.4, and 9.6 Å. The underlying structure was identified as a four-stranded  $\beta$ -sheet core in a cross- $\beta$  arrangement. Interestingly, these prion strains vary substantially in their biological properties, e.g., incubation times (from ~75 to ~600 d) and proteinase K resistance levels (resistant, intermediate, and fully sensitive), but still share a common core structure.

Electron microscopy was used again to determine the diameters of individual amyloid fibrils. As before, the measured average diameters fell into a narrow range of 48 to 60 Å. The relatively homogeneous fibril diameters confirm the compact nature of the  $\beta$ -sheet structure and exclude more extended  $\beta$ -sheet folds; for instance, a parallel in-register  $\beta$ -sheet structure would result in a substantially wider fibril diameter than what was observed in the electron micrographs.

In summary, combining data obtained by X-ray fiber diffraction and electron microscopy on 16 different prion isolates (natural, synthetic, and mutant prion protein strains) allowed us to determine a feature that may be common to most, if not all, mammalian prion strains: a compact, four-stranded  $\beta$ -sheet core in a cross- $\beta$  configuration.

### OR-04: Structure and dynamics of a toxic Syrian hamster prion protein $\beta$ -intermediate

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The formation of specific  $\beta$ -structured, non-fibrillar, intermediates during misfolding pathway of the mammalian prion protein has been shown to correlate with prion disease susceptibility.<sup>1</sup> Thus, this  $\beta$ -state is anticipated to play an important role in prion disease pathology, during infection with PrP<sup>Sc</sup> and in cell death during neurodegeneration. Despite the potential role of non-fibrillar PrP assemblies in prion disease, little is known about their molecular structure, and obtaining a structural model for these oligomeric intermediates will provide a better understanding of their relationship with PrP<sup>Sc</sup>, and assist in determining their role in the pathogenesis of prion diseases.

We are using a combined biophysical and structural approach to obtain a detailed characterization of the molecular structure and dynamic behavior of stable  $\beta$ -state oligomers formed during misfolding of the Syrian hamster prion protein (ShPrP). Previous reports have shown that these oligomers exhibit cytotoxicity in neuronal cell culture, and are closely related to those formed by other mammalian PrP.<sup>1</sup> Here we present the first molecular level

structural information for a non-fibrillar  $\beta$ -state PrP oligomer, and shed new light on the mechanism of PrP conversion.

Using a combination of single particle electron microscopy, atomic force microscopy and small-angle X-ray scattering, we have shown that the  $\beta$ -state oligomers are octameric, with a flattened discoidal morphology whose 4-fold symmetry is most consistent with a tetramer of dimers. Solid state NMR measurement of select intermolecular  $^{13}\text{C}$ - $^{13}\text{C}$  distances has identified intermolecular parallel  $\beta$ -sheets within the structurally ordered core of the octamers, suggestive of the strand arrangement observed in previous studies of amyloid fibrils formed by PrP.<sup>2,3</sup> In contrast to fibrils, the  $\beta$ -state oligomers exhibit significant internal dynamics, and also require the presence of the disordered N-terminus for stability. These data are summarized in a structural model for the  $\beta$ -state oligomers which provides new insight into molecular rearrangements that occur early in PrP misfolding.

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### OR-05: Conformation selective prion amplification using specific shear fields

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Aging-related human neurodegenerative diseases are associated with the progressive conversion of disease-specific proteins into toxic conformational variants. Once formed, these conformational variants spread to neighboring cells by an infection-like process. This mechanism of propagation is well established in the case of mammalian prions, and there is mounting evidence that also Alzheimer disease and possibly also Parkinsons disease may follow prion-like mechanisms of systemic spreading.

For prion diseases the molecular properties of the native polypeptide chains have been extensively investigated by NMR-spectroscopy (e.g., see ref. 1). Some insight into fibrillar structures has for example been obtained by H/D exchange NMR.<sup>2</sup> However, overall there is very limited quantitative knowledge about the disease-specific, in vivo active conformational variants. This can be attributed to two main challenges: First, the structural investigation of aggregated protein conformations still represents a substantial biophysical challenge. Second, it is generally non-trivial to amplify specific and homogeneous conformations of proteopathic aggregates in vitro. To address this problem, we have developed novel technologies for the in vitro amplification of mammalian prions.

It had been shown by others<sup>3,4</sup> that infectious prions can be amplified in vitro by the PMCA method using repeated cycles ultrasonic fragmentation and incubation. While we were able to reproduce key results using 263K hamster prions, in our hands it turned out to be unexpectedly hard to adapt PMCA to other prion strains or to even detect prion strains in a diagnostic-like setting. We therefore investigated in detail the physical basis of prion in vitro amplification. We found that the selective amplification of prions requires shear fields that have to be controlled within very narrow margins for strain specific fragmentation. Furthermore, the efficiency of amplification was also strongly influenced by the duration of the shearing, and the cycle time. Under optimal parameters, prion amplification was observed to become highly efficient so that the half maximal amplification (approx. 40x) could be achieved in only 6 h. While our approach also worked using specific ultrasonic shearing, most of the data to be presented here was accumulated using a mechanical shearing device that we developed for the parallel and specific processing of multiple samples.

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### OR-06: Prion diversity and evolution—What animal strain typing tells us

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Mammalian prions are primarily composed of PrP<sup>Sc</sup>, a misfolded, multimeric form of the host-encoded cellular prion protein (PrP<sup>C</sup>). Prions share many properties with conventional infectious agents, including the existence of multiple prion strains in the same host, the capacity to propagate between species and to evolve by mutation and/or selection under selective pressure. Prion strain diversity is assumed to reflect the existence of stable, structurally distinct PrP<sup>Sc</sup> conformers, at the level of the tertiary and/or quaternary structure. The so-called 'species barrier' that limits the cross-species transmission of prions is thought to depend on the conformational compatibility between host PrP<sup>C</sup> and the infecting PrP<sup>Sc</sup>. Due to missing ultrastructural methods of analysis, studying such events requires transmission to laboratory animals and analysis of prion-specific phenotypic traits. Then the question arises of how much information might be lost between the initial stages of the infection and the terminal phase at which such typing is performed.

Historically, conventional mouse models have been instrumental to develop robust strain typing methods aimed at classifying animal prion strains. However, in many instances, their susceptibility to disease was limited by the species barrier phenomenon, resulting in poor transmission and/or strain modification. Transgenic mouse models have permitted to circumvent this problem through the expression of the sole prion protein of concern. Despite tremendous progress in the diversity of prion-affected species typed and in the characterization of newly recognized strains, notably in ruminants, such models have also revealed a disconcerting divergent capacity of evolution of prions, even within the same species or in different tissues of the same species. To explain these phenomena, it has been proposed that prions may be endowed with a variety of PrP<sup>Sc</sup> conformers, the fittest conformation being selected in a particular environment or tissue. The next challenges will be to analyze in 'real time' such molecular events and to decipher further the contribution of the cell environment to such process.

### OR-07: PrP<sup>Sc</sup> profiles in TSE strain typing

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From early days, the phenotypical behavior of TSE strains has been characterized by mouse bio-assay in which inbred mice are inoculated intracerebrally with TSE isolates. The combination of the incubation period and the profile of the vacuolar lesions in the brains of these mice is then used to identify the TSE strain involved. There are some major drawbacks to the bio-assay in mice: (1) the incubation period can only be determined once it has stabilized which often requires further subpassages in mice; (2) vacuolation can occur spontaneously in the brain of elderly control mice; (3) vacuolation can be minimal in some TSE strains in mice; and (4) individual mouse vacuolation profiles do not give information on the TSE strain type since they are not identical to the average profile due to variation between mice.

In the Netherlands, TSE strain typing facilities have been set up since 2004 using VM, RIII, Tg338 and Tg110 mice. In order to bypass the problems associated with vacuolation profiles, we used a scanning light microscopy system to create high resolution PrP<sup>Sc</sup> profiles based on the immunohistochemical detection of PrP<sup>Sc</sup> in both coronal and sagittal sections of the mouse brain.

First, a reference frame work has been set up by determining the PrP<sup>Sc</sup> profiles of a number of TSE reference strains (87A, 87V, 301C, 301V, 79 A, 79V, 22A, 22C, ME7). Then a number of TSE isolates (classical and atypical scrapie, L-, C- and H- type BSE) from Dutch and other European sources have been strain typed and PrP<sup>Sc</sup> lesion profiles were compared with the TSE reference strains. Interim results will be presented in which we will show that PrP<sup>Sc</sup> profiles can be used to type TSE strains in individual mice after primary passage.

### OR-08: Strain-specific role of RNAs in prion replication

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The prevalent hypothesis in prion diseases proposes that the infectious agent is mainly or exclusively composed of a misfolded version of the cellular prion protein. Several lines of evidence suggest that other co-factors may also be required for prion replication. PrP binds to polyanions such as RNAs and glycosaminoglycans and recently RNAs were shown to promote and induce the conversion of PrP<sup>C</sup> into PrP<sup>Sc</sup> in vitro. In the present study, we used the serial automated Protein Misfolding Cyclic Amplification (saPMCA) and the mouse bioassay to investigate in more detail the role of RNAs in prion replication.

We found that RNase treatment impairs PrP<sup>Sc</sup> converting activity in a strain-specific fashion. Interestingly we observed that in vitro conversion of ME7 was fully dependent on the presence of RNAs while replication of RML could persist in their absence. Moreover, we found that, whereas ME7 amplification could be reconstituted only by addition of RNA, RML conversion was restored and even enhanced by different polyanionic molecules.

In an attempt to determine whether RNA confers strain-specific characteristics to prions, we evaluated the biological properties of RML propagated by PMCA in the absence of RNA molecules (RML-PRNaseA) and compared them to brain-derived (RML-B) and PMCA material generated in the presence of RNA (RML-P and RML-PRNaseOut). Transmissions were performed in wild-type and Tga20 mice; secondary transmissions were performed from the latter. Interestingly, inoculation of RML-PRNaseA resulted in a distinctive disease phenotype characterized by fore limb paresis in 50% of Tga20 mice inoculated. This new phenotype was not conserved after secondary transmission. In wild-type mice, RML-P and RML-PRNaseA inocula produced classical signs of prion disease and similar incubation times. Titration in Tga20 mice showed that, while the specific infectivity per PrP<sup>Sc</sup> molecule of RML-PRNaseOut was similar to brain-derived RML, RML-PRNaseA was 3.5 times more infectious. This difference however is not large enough to be attributed to the generation of a new strain. Immunohistochemical analysis of RML-PRNaseA, RML-PRNaseOut and RML-P inoculated mouse brains did not reveal any differences in vacuolation and PrP<sup>Sc</sup> accumulation patterns suggesting that RML replication in the absence of RNA did not modify the strain. This was confirmed in vitro by comparing cell tropism of prions from RML-PRNaseA, RML-PRNaseOut and RML-P infected mouse brains.

We conclude that RNA-requirement for in vitro conversion is prion strain-dependent, and that replication under RNA-depleted conditions did not modify RML prion strain properties. Our study cannot, however, exclude small variations of RML properties that would explain the new clinical phenotype



observed. We stipulate that RNA molecules may act as strain-specific catalysts of the prion replication process.

## OR-09: Canine spongiform encephalopathy—A new form of animal prion disease

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It was also hypothesized that BSE might have originated from an unrecognized sporadic or genetic case of bovine prion disease incorporated into cattle feed or even cattle feed contaminated with prion-infected human remains.<sup>1</sup> However, strong support for a genetic origin of BSE has recently been demonstrated in an H-type BSE case exhibiting the novel mutation E211K.<sup>2</sup> Furthermore, a specific prion protein strain causing BSE in cattle is believed to be the etiological agent responsible for the novel human prion disease, variant Creutzfeldt-Jakob disease (vCJD).<sup>3</sup> Cases of vCJD have been identified in a number of countries, including France, Italy, Ireland, the Netherlands, Canada, Japan, US and the UK with the largest number of cases. Naturally occurring feline spongiform encephalopathy of domestic cats<sup>4</sup> and spongiform encephalopathies of a number of zoo animals so-called exotic ungulate encephalopathies<sup>5,6</sup> are also recognized as animal prion diseases, and are thought to have resulted from the same BSE-contaminated food given to cattle and humans, although and at least in some of these cases, a sporadic and/or genetic etiology cannot be ruled out. The canine species seems to display resistance to prion disease and no single case has so far been reported.<sup>7,8</sup> Here, we describe a case of a 9 week old male Rottweiler puppy presenting neurological deficits; and histological examination revealed spongiform vacuolation characteristic of those associated with prion diseases.<sup>9</sup> Initial biochemical studies using anti-PrP antibodies revealed the presence of partially proteinase K-resistant fragment by western blotting. Furthermore, immunohistochemistry revealed spongiform degeneration consistent with those found in prion disease and displayed staining for PrP<sup>Sc</sup> in the cortex.

Of major importance, PrP<sup>Sc</sup> isolated from the Rottweiler was able to cross the species barrier transmitted to hamster in vitro with PMCA and in vivo (one hamster out of 5). Furthermore, second in vivo passage to hamsters, led to 100% attack rate (n = 4) and animals displayed atypical lesional profile and shorter incubation period.

In this study, we show that the canine species might be sensitive to prion disease and that PrP<sup>Sc</sup> isolated from a dog can be transmitted to dogs and hamsters in vitro using PMCA and in vivo to hamsters.

If our preliminary results are confirmed, the proposal will have a major impact on animal and public health and would certainly lead to implementing new control measures for 'canine spongiform encephalopathy' (CSE).

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## OR-10: Variably protease-sensitive prionopathy is transmissible in bank voles

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**Background.** Variably protease-sensitive prionopathy (VPSPr) is a recently described "sporadic" neurodegenerative disease involving prion protein aggregation, which has clinical similarities with non-Alzheimer dementias, such as fronto-temporal dementia. Currently, 30 cases of VPSPr have been reported in Europe and USA, of which 19 cases were homozygous for valine at codon 129 of the prion protein (VV), 8 were MV and 3 were MM. A distinctive feature of VPSPr is the electrophoretic pattern of PrP<sup>Sc</sup> after digestion with proteinase K (PK). After PK-treatment, PrP from VPSPr forms a ladder-like electrophoretic pattern similar to that described in GSS cases. The clinical and pathological features of VPSPr raised the question of the correct classification of VPSPr among prion diseases or other forms of neurodegenerative disorders. Here we report preliminary data on the transmissibility and pathological features of VPSPr cases in bank voles.

**Materials and Methods.** Seven VPSPr cases were inoculated in two genetic lines of bank voles, carrying either methionine or isoleucine at codon 109 of the prion protein (named BvM109 and BvI109, respectively). Among the VPSPr cases selected, 2 were VV at PrP codon 129, 3 were MV and 2 were MM. Clinical diagnosis in voles was confirmed by brain pathological assessment and western blot for PK-resistant PrP<sup>Sc</sup> (PrP<sup>res</sup>) with mAbs SAF32, SAF84, 12B2 and 9A2.

**Results.** To date, 2 VPSPr cases (1 MV and 1 MM) gave positive transmission in BvM109. Overall, 3 voles were positive with survival time between 290 and 588 d post inoculation (d.p.i.). All positive voles accumulated Pr<sup>P<sub>res</sub></sup> in the form of the typical PrP<sup>27-30</sup>, which was indistinguishable to that previously observed in BvM109 inoculated with sCJDMM1 cases.

In BvI109, 3 VPSPr cases (2 VV and 1 MM) showed positive transmission until now. Overall, 5 voles were positive with survival time between 281 and 596 d.p.i.. In contrast to what observed in BvM109, all BvI109 showed a GSS-like PrP<sup>Sc</sup> electrophoretic pattern, characterized by low molecular weight PrP<sup>P<sub>res</sub></sup>. These PrP<sup>P<sub>res</sub></sup> fragments were positive with mAb 9A2 and 12B2, while being negative with SAF32 and SAF84, suggesting that they are cleaved at both the C-terminus and the N-terminus. Second passages are in progress from these first successful transmissions.

**Conclusions.** Preliminary results from transmission studies in bank voles strongly support the notion that VPSPr is a transmissible prion disease. Interestingly, VPSPr undergoes divergent evolution in the two genetic lines of voles, with sCJD-like features in BvM109 and GSS-like properties in BvI109.

The discovery of previously unrecognized prion diseases in both humans and animals (i.e., Nor98 in small ruminants) demonstrates that the range of prion diseases might be wider than expected and raises crucial questions about the epidemiology and strain properties of these new forms. We are investigating this latter issue by molecular and biological comparison of VPSPr, GSS and Nor98.

### OR-11: Plasmacytoid dendritic cells sequester high prion titers at early stages of prion infection

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In most transmissible spongiform encephalopathies prions accumulate in the lymphoreticular system (LRS) long before they are detectable in the central nervous system. While a considerable body of evidence showed that B lymphocytes and follicular dendritic cells play a major role in prion colonization of lymphoid organs, the contribution of various other cell types, including antigen-presenting cells, to the accumulation and the spread of prions in the LRS are not well understood. A comprehensive study to compare prion titers of candidate cell types has not been performed to date, mainly due to limitations in the scope of animal bioassays where prohibitively large numbers of mice would be required to obtain sufficiently accurate data. By taking advantage of quantitative in vitro prion determination and magnetic-activated cell sorting, we studied the kinetics of prion accumulation in various splenic cell types at early stages of prion infection. Robust estimates for infectious titers were obtained by statistical modeling using a generalized linear model. While prions were

detectable in B and T lymphocytes and in antigen-presenting cells like dendritic cells and macrophages, highest infectious titers were determined in two cell types that have previously not been associated with prion pathogenesis, plasmacytoid dendritic (pDC) and natural killer (NK) cells. At 30 d after infection, NK cells were more than twice, and pDCs about 7-fold, as infectious as lymphocytes respectively. This result was unexpected since, in accordance to previous reports prion protein, an obligate requirement for prion replication, was undetectable in pDCs. This underscores the importance of prion sequestration and dissemination by antigen-presenting cells which are among the first cells of the immune system to encounter pathogens. We furthermore report the first evidence for a release of prions from lymphocytes and DCs of scrapie-infected mice ex vivo, a process that is associated with a release of exosome-like membrane vesicles.

### OR-12: Chronic wasting disease transmission and pathogenesis in cervid and non-cervid Species

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Since its recognition as a TSE in the late 1970s, chronic wasting disease (CWD) of cervids has been distinguished by its facile spread and is now recognized in 18 states, 2 Canadian provinces, and South Korea. The efficient horizontal spread of CWD reflects a prion/host relationship that facilitates efficient mucosal uptake, peripheral lymphoid amplification, and dissemination by exploiting excretory tissues and their products, helping to establish indirect/environmental and well as direct (e.g., salivary) transmission. Recent studies from our group also support the likelihood of early life mother to offspring and aerosol CWD prion transmission. Studies of cervid CWD exposure by natural routes indicate that incubation period for detection of overt infection, while still uncertain, may be much longer than originally thought.

Several non-cervid species can be infected by CWD experimentally (e.g., ferrets, voles, cats) with consequent species-specific disease phenotypes. The species-adapted prions so generated can be transmitted by mucosal, i.e., more natural, routes. Whether non-cervid species sympatric with deer/elk can be infected in nature, however, remains unknown. In vitro CWD prion amplification studies, in particular sPMCA, can foreshadow in vivo susceptibility and suggest the importance of the PrP<sup>C</sup> rigid loop region in species barrier permissiveness. Trans-species CWD amplification appears to broaden the host range/strain characteristics of the resultant prions. The origins of CWD remain unknown, however, the existence of multiple CWD prion strains/quasi-species, the mechanisms of prion shedding/dissemination, and the relationship between sheep scrapie and CWD merit further investigation.

### OR-13: Sustained translational repression by eIF2 $\alpha$ -P mediates prion neurodegeneration

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The mechanisms leading to neuronal death in neurodegenerative disease are poorly understood. Many of these disorders, including Alzheimer (AD), Parkinson (PD) and prion diseases, are associated with the accumulation of misfolded disease-specific proteins. The unfolded protein response (UPR) is a protective cellular mechanism triggered by rising levels of misfolded proteins. One arm of this pathway results in the transient shutdown of protein translation, through phosphorylation of the  $\alpha$  subunit of eukaryotic translation initiation factor, eIF2 $\alpha$ . UPR activation and/or increased eIF2 $\alpha$ -P levels are seen in patients with AD, PD and prion disease<sup>1-4</sup> but how this links to neurodegeneration is unknown. Here we show that accumulation of prion protein (PrP) during prion replication causes persistent translational repression of global protein synthesis by eIF2 $\alpha$ -P, with abrupt loss of synaptic proteins, associated with synaptic failure and neuronal loss in prion-diseased mice. Further, we show that promoting translational recovery in hippocampi of prion-infected mice is neuroprotective. Overexpression of GADD34, a specific eIF2 $\alpha$ -P phosphatase, as well as reduction of PrP levels by lentivirally-mediated RNAi, reduced eIF2 $\alpha$ -P levels. As a result, both approaches restored vital translation rates during prion disease, rescuing synaptic deficits and neuronal loss, and thereby significantly increasing survival. In contrast, salubrinal, an inhibitor of eIF2 $\alpha$ -P dephosphorylation<sup>5</sup> increased eIF2 $\alpha$ -P levels, exacerbating neurotoxicity and significantly reducing survival in prion diseased mice. Given the prevalence of protein misfolding and UPR activation in several neurodegenerative diseases, our results suggest that manipulation of common pathways such as translational control, rather than disease-specific approaches, may lead to new therapies preventing synaptic failure and neuronal loss across the spectrum of these disorders.

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### OR-14: Early plasma membrane accumulations of nascent prions in hippocampus—Possible sites of replication, cell-to cell transfer and pathology

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The subcellular site of PrP<sup>C</sup> to PrP<sup>Sc</sup> conversion in prion-infected brain, the origin of neuropathology and the means of intercellular PrP<sup>Sc</sup> spread have not yet been established. Here, we used high resolution immunofluorescence, cryo-immunogold electron microscopy and tomography to investigate these three aspects of prion disease in mice infected intracerebrally with RML prions.

We used the F4-31 antibody to detect PrP<sup>C</sup> specifically.<sup>1</sup> After PrP<sup>C</sup> to PrP<sup>Sc</sup> conversion, PrP<sup>Sc</sup> may undergo N-terminally trimming.<sup>2</sup> We therefore used Saf32, which binds to the N-terminus of PrP, to detect PrP<sup>C</sup> and recently formed PrP<sup>Sc</sup> in hippocampus *stratum oriens* during the course of prion disease. By comparing antibody labeling patterns in uninfected and infected brains, we showed that clusters of Saf32 cryo-immunogold EM labeling and foci of increased immunofluorescence indicate sites containing PrP<sup>Sc</sup>. PrP<sup>Sc</sup> was already detectable at the earliest stage examined, 65 d post inoculation, or 54% of the incubation period to clinical disease, and its prevalence increased during disease progression.

More than 80% of Saf32 immunogold labeling clusters were found on neuronal plasma membranes, most strikingly on membrane invaginations and sites of cell-to-cell contact. Both axons and dendrites were affected, and clustered labeling was occasionally found at synapses. We suspect that prion conversion occurs mainly on plasma membranes close to nerve terminals, where intercellular prion transfer may also occur. Neuropathology may also be initiated at these plasma membranes.

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### OR-15: Advances in the genetic epidemiology of Creutzfeldt-Jakob disease and related diseases

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Creutzfeldt-Jakob disease (CJD) is a rare transmissible neurodegenerative disorder. The role of genetic factors in its aetiology

has been recognized for long but is far from understood. An important determinant for CJD is the M129V polymorphism of the human prion protein gene (PRNP). Both experimental and observational studies suggest that there are also other coding and non-coding genetic polymorphisms inside and outside this gene relevant, including the PRNP regulatory region. Within the EUROCCJD surveillance network, we have conducted a series of genomic studies to improve our understanding of the genes involved in various form of human CJD, in particular sporadic (sCJD) and variant CJD (vCJD). Our studies support the hypothesis that genetic variations in the PRNP promoter may have a role in the pathogenesis of sCJD, suggesting that expression of PRNP may be relevant. However, we did not find evidence of duplications of PRNP. We further conducted genome wide association studies (GWAS) of both sCJD and vCJD. Our studies in 135 vCJD patients of the UK and France suggest that genetic variations in phosphatidylinositol pathway enzymes are associated to vCJD susceptibility. These findings may be explained by the fact that these are linked to functional changes hampering protein function. Dysfunctional phospholipases have been suggested to increase the availability of PrPC at neuronal cell membranes facilitating PrPSc propagation. Results in our ongoing GWAS study of vCJD point towards a very different pathway involving genes of which the protein products are interacting directly with PRNP and 14-3-3 proteins. GWAS findings were compared with the expression data available at the <http://prion.systemsbiology.net>. This data base shows an extensive number of genes involved in Alzheimer's disease that are differentially expressed during different stages of prion infection. This finding is in line with familial aggregation of CJD and other neurodegenerative diseases including Alzheimer's disease and raises the question whether the protein aggregations seen in Alzheimer's and Parkinson's disease can be explained by the same genetic mechanisms as seen in CJD. Genetically CJD is a unique disease in that there is a reduction of heterozygosity at the gene (PRNP). We have tested the hypothesis whether also for Alzheimer's disease and Parkinson's disease there is evidence for reduced heterozygosity of genes encoding the protein that is aggregating in the brain. These studies were conducted within the Rotterdam study, a population-based study of 11,800 persons aged 55 years or older. For neither Alzheimer's disease nor Parkinson's disease there was evidence for such mechanism for the key proteins. Excess homozygosity was seen in Alzheimer's disease in a small subgroup of patients. A more extensive study involving larger protein pathways is still ongoing and will be presented.

## OR-16: A cost effectiveness study of the use of ante-mortem TSE tests on cattle in the United Kingdom

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This paper presents the results of a study to assess the cost effectiveness of the introduction of ante-mortem tests for Transmissible Spongiform Encephalopathies (TSEs) in the United Kingdom. The study focused on the implementation of ante-mortem tests for BSE in cattle at the current stage of the BSE epidemic, when prevalence levels are low.

A set of possible scenarios for the introduction of live animal tests for TSEs was developed and reviewed at an Expert Workshop involving experts from a range of stakeholders, including regulators, the meat industry, veterinarians and people involved in test development. This review concluded that the cost effectiveness assessment should focus on the introduction of a live test on healthy cattle performed on farm before being sent for slaughter and involving either all cattle sent for slaughter or just adult cattle (over 48 mo old at slaughter) as currently tested post-mortem.

The cost effectiveness of the introduction of an ante-mortem TSE test was evaluated by considering the range of costs that would be associated with implementing ante-mortem testing for the agreed scenarios compared with the benefit, measured as the estimated change in exposure to TSE infectivity resulting from the use of the test. The potential for TSE infectivity to enter the human food supply was evaluated using a TSE Exposure model based on the DNV SRM Controls Model that had been previously developed for the UK Food Standards Agency and used to estimate the impact of alternative supervision strategies for SRM Controls. Overall cost effectiveness for each test scenario was then presented as the estimated reduction in bovine oral ID<sub>50</sub> units per million pounds of cost. A probabilistic risk model was developed and evaluated using @RISK software.

Assuming that an ante-mortem test would be able to detect infectivity for a wider range of the incubation period than is currently possible with the present post-mortem tests, at least for the last 12 mo of the incubation period, then it was estimated that the median exposure would reduce from 3 bovine oral ID<sub>50</sub> units (for the whole beef eating population of the UK) with the present post-mortem testing program (range 0.2 to 52) to 0.02 bovine oral ID<sub>50</sub> units (range < 0.01 to 0.5) with the ante-mortem test applied only to animals older than 48 mo slaughtered for food and a test with 100% sensitivity. The cost effectiveness of the ante-mortem test applied to over 48 mo animals was estimated to be 2.1 bovine oral ID<sub>50</sub> units per £ million spent as opposed to 0.9 for the post-mortem test. However, the fact that the TSE exposure to the UK population from beef consumption is already at a very low level should be taken into account when assessing the significance of this increase in cost effectiveness.

The study included a sensitivity assessment for key variables, including the cost of an ante-mortem test.



## OR-17: Old cattle and the risk of atypical bovine spongiform encephalopathy

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**Introduction.** H and L-types of atypical Bovine Spongiform Encephalopathy (BSE) were identified in 2003 in Italy and France respectively before being identified in other countries worldwide. Around 60 atypical BSE cases have currently been reported in 13 countries, with over one third in France. While the epidemiology of classical BSE has been widely described, atypical BSEs are still poorly documented, but appear to differ from classical BSE.

**Material and Methods.** We analyzed the epidemiological characteristics of the 12 cases of L-type and 11 cases of H-type of BSE detected in France from January 2001 to December 2009 and looked for individual risk factors. As L-type of BSE cases did not appear homogeneously distributed throughout the country, two complementary methods were used: spatial analysis and regression modeling. L- and H-types were studied separately as both the biochemical properties of their pathological prion protein and their features differ in animal models.

**Results.** More than 80% of the L and H atypical BSE cases were detected in beef cattle, whatever the atypical type, while classical BSE concerned mainly dairy cattle. The average age at detection for L- and H-type cases was 13.0 and 12.4 y respectively, with no difference between the two types. However, this average age differed significantly from that of classical BSE (7.3 y). A significant geographical cluster was detected for L-type BSE. Among animals over eight years old, we showed that the risk of L-type of BSE increased with age. This was not evidenced for H-type of BSE.

**Discussion.** This is the first time that a study has addressed the epidemiology of the two types of atypical BSE. The geographical cluster evidenced for L-type of BSE could be partly due to the age structure of the background-tested bovine population. The model, adjusted for the age and birth cohort, evidenced an age effect for L-type of BSE and the descriptive analysis showed a particular age structure in the area where the cluster was detected. No birth cohort effect was revealed. Such findings are coherent with the high number of L-type of BSE cases detected in Poland where the proportion of animals tested over eight years old is one of the highest in Europe. However, the small number of cases of atypical BSE included in the study and the few individual data available limited the analysis to the investigation of age and cohort effect only. In this context, it appears essential to maintain the surveillance of BSE to consolidate our initial findings and improve knowledge of these diseases.

## OR-18: The nature and significance of protein aggregation phenomena

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Natural proteins are a highly select group of molecules, and their properties have a number of very special characteristics when compared with random sequences of amino acids, one of which is the ability to fold to unique and often highly intricate structures, a characteristic that has enabled biological systems to generate a vast range of functions and an astonishing degree of specificity in their chemical processes. Proteins are, however, prone to aggregation and in some cases this phenomenon gives rise to highly ordered structures with remarkable stability and previously unexpected properties. This lecture will describe the underlying nature of the protein aggregation phenomenon and in particular how it can give rise to a wide range of diseases that have become of major significance in the modern world.

## OR-19: Synthetic PrP related disorders and natural TSEs share disease associated PrP membrane accumulation and pathology but differ in within brain dissemination pathways

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The light microscopic lesions of prion diseases: spongiosis, neuronal loss and disease associated PrP (PrP<sup>d</sup>) accumulation, are well known, but distinctive sub-cellular changes are also found. Some specific sub-cellular lesions of naturally occurring TSEs co-localize with PrP<sup>d</sup> but others do not.

In the naturally occurring animal TSEs and their rodent models, PrP<sup>d</sup> accumulates on the plasmalemma of neurons and glia. This accumulation initially occurs in the absence of any other changes suggesting that the conversion of normal PrP (PrP<sup>C</sup>) to PrP<sup>d</sup> occurs at this site. PrP<sup>d</sup> co-localizes with specific plasmalemmal changes that includes an increase in coated pits, coated and ubiquitinated spiral membrane invaginations on dendrites and axons and membrane microfolding. Ubiquitinated membrane PrP<sup>d</sup> may be translocated from the plasma-lemma to lysosomes where it is truncated.

The same neuritic membrane changes are present in scrapie infected transgenic rodent lines in which PrP<sup>C</sup> is confined to astrocytes but are absent in scrapie infected GPI anchorless mice even though abundant amyloid plaques may be formed in these latter mice. These data show that PrP<sup>d</sup> can be transferred from one membrane to another and that membrane toxicity requires PrP<sup>d</sup> to remain anchored to the membrane. However, aggregation of PrP<sup>d</sup> into fibrils appears to be facilitated by release from membranes to the extracellular space suggesting that conversion and aggregation occur as separate processes.

Synthetic PrP seeds or brain homogenates derived from their sub-passage, have been able to induced clinical disease, neuropil vacuolation and abnormal PrP (PrP<sup>syn</sup>) accumulation following their intra-cerebral inoculation into rodents. Synthetic PrP seeds at primary passage lack a GPI anchor but are nevertheless able to induce conversion of PrP<sup>c</sup> at cell membranes and induce extracellular amyloid plaque formation but lack PrP<sup>d</sup> related membrane changes. Rodent passaged synthetic PrP seeds, which presumptively possess GPI anchors, are able to convert membrane PrP<sup>c</sup>, induce amyloid plaques and also show membrane-PrP<sup>syn</sup> associated lesions that are indistinguishable from those found in natural TSEs. However, PrP<sup>syn</sup> does not internalise to neuronal or astroglial lysosomes, does not recapitulate all of the non-PrP<sup>d</sup> linked lesions seen in natural TSEs and may occlude interstitial fluid drainage pathways and cause cerebrovascular amyloid angiopathy. These data show that PrP<sup>syn</sup> may be sufficient to convert membrane PrP<sup>c</sup> and to create seeds that initiate amyloidosis but may not reproduce all aspects of TSE neuropathology.

The nature of the PrP<sup>syn</sup> and its passage history and the genetics of the host are all important factors in determining whether the PrP<sup>syn</sup> amplifies in recipients and the extent of the lesions caused. However, end-stage neuropathology appears to be strongly influenced by the physical distribution of the inoculum, recirculation, and interstitial fluid drainage pathways.

## OR-20: RNA-binding proteins with prion-like domains in ALS and beyond

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Prions are self-templating protein conformers that are naturally transmitted between individuals and promote phenotypic change. In yeast, prion-encoded phenotypes can be beneficial, neutral or deleterious depending upon genetic background and environmental conditions. A distinctive and portable 'prion domain' enriched in asparagine, glutamine, tyrosine and glycine residues unifies the majority of yeast prion proteins. Deletion of this domain precludes prionogenesis and appending this domain to reporter proteins can confer prionogenicity. An algorithm designed to detect prion domains was used to scour the human genome and revealed a select group of RNA-binding proteins harboring a canonical RNA recognition motif (RRM) and a putative prion domain. Startlingly, these RNA-binding prion candidates are inexorably emerging, one by one, in the pathology and genetics of devastating neurodegenerative disorders, including: frontotemporal lobar degeneration with ubiquitin-positive inclusions (FTLD-U) and amyotrophic lateral sclerosis (ALS). For example, FUS and TDP-43, which rank 1st and 10th among RRM-bearing prion candidates, form cytoplasmic inclusions in the degenerating motor neurons of ALS patients and mutations in TDP-43 and FUS cause familial ALS. Recently, perturbed RNA-binding proteostasis of TAF15, which is the 2nd ranked RRM-bearing prion candidate, has been connected with

ALS and FTLD-U. Here, I will discuss additional RNA-binding prion candidates identified by our algorithm that are surfacing as genetic modifiers or causes of diverse neurodegenerative conditions. Indeed, simple prion-like transfer mechanisms involving the prion-like domains of RNA-binding proteins could underlie the classical non-cell-autonomous emanation of neurodegenerative pathology from originating epicenters to neighboring portions of the nervous system.

## OR-21: Prion-seeded conversion of recombinant PrP: implications for prion biology and diagnostics

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TSE-associated forms of PrP have long been known to induce specific conformational changes in normal PrP<sup>Scn</sup>. This activity serves not only as the core event in prion propagation, but also as the basis for highly sensitive tests for TSE infections. The use of bacterially expressed recombinant PrP<sup>Scn</sup> (rPrP<sup>Scn</sup>) as a substrate for prion seeded conversion has allowed the generation of infectious prions from defined biochemical components, e.g. ref 1. Moreover, development of reactions employing this economical and relatively abundant substrate has promoted the speed and practicality of assays for prion seeding activity. Two recent manifestations of such assays, real-time quaking-induced conversion QuIC (RT-QuIC)<sup>2,3</sup> and enhanced QuIC (eQuIC),<sup>4</sup> can allow relatively high-throughput, sensitive and quantitative detection of prion seeding activity in a variety of samples, including dilute and/or inhibitor-laden fluids such as blood plasma,<sup>4</sup> cerebral spinal fluid (CSF)<sup>2,3,5</sup> and nasal fluids.<sup>6,7</sup> Notably, others have shown that RT-QuIC has shown promise for improving the antemortem diagnosis of human sCJD using cerebral spinal fluid samples, e.g. ref 2. Quantitative RT-QuIC analyses of the kinetics of appearance of prion seeding activity in the CSF of scrapie-infected hamsters showed striking dependence on the route of inoculation (intracerebral vs intratongue).<sup>5</sup> Further applications of RT-QuIC and eQuIC assays to variety of prions and sample types are in progress.

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## OR-22: Propagated misfolding of SOD1 in ALS

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Approximately 10% of ALS cases are familial, with ~20% of these due to mutations in the gene encoding Cu/Zn superoxide dismutase (SOD1), a ubiquitous free-radical defense enzyme. A consequence of SOD1 mutation and/or oxidation is a propensity of the protein to misfold and aggregate. Human wild-type (wt) SOD1 is known to co-aggregate with mutant SOD1 in familial ALS, in double transgenic mouse models, and in cell culture systems, but the structural determinants of this process and its functional consequences are unclear. We sought to molecularly dissect the effects of intracellular obligately misfolded SOD1 mutant proteins on natively structured wild-type SOD1. Expression of the enzymatically inactive, natural familial ALS SOD1 mutations G127X and G85R in human mesenchymal and neural cell lines induced misfolding of wild-type natively-structured SOD1, as indicated by: (1) acquisition of immunoreactivity with SOD1 misfolding-specific monoclonal antibodies; (2) markedly enhanced protease sensitivity suggestive of structural loosening; and (3) non-native disulfide-linked oligomer and multimer formation. Cytosolic mislocalizing mutations of FUS and TDP43, two proteins implicated in familial and sporadic ALS, also triggered SOD1 misfolding. Expression of G127X and G85R in mouse cell lines did not induce misfolding of murine wtSOD1, and a species restriction element for human wtSOD1 conversion was mapped to a region of sequence divergence in loop II and  $\beta$ -strand 3 of the SOD1  $\beta$ -barrel (residues 24–36), then further refined surprisingly to a single tryptophan residue at codon 32 in human SOD1. Aggregated recombinant G127X is capable of inducing misfolding of recombinant human wtSOD1 in a cell-free system in buffered saline containing reducing and chelating agents. The presence of a tryptophan at codon 32 on recombinant G127X increases its effectiveness at inducing wtSOD1 misfolding, compared with a serine at position 32 as found in the murine SOD1 sequence. Culture medium from cells transiently transfected with wild-type or mutant SOD1 induced misfolding of endogenous SOD1 when added to naive neuroblastoma cell cultures, and this process was stably propagated in serial passage. Nonspecific uptake of misfolded SOD1 was excluded by siRNA knockdown of SOD1 in the fresh recipient cells, indicating a requirement for endogenously expressed SOD1 as a

substrate. The agent responsible for induction of misfolding was determined to be a misfolded SOD1 aggregate which pelleted by ultracentrifugation of 100,000 X g for 1 h. Transmission of SOD1 misfolding in vitro was abrogated by extracellular pan- and misfolding-specific SOD1 antibodies. G37R Tg mice treated with misfolding-specific SOD1 antibodies displayed prolonged survival of ~11 d ( $p < 0.001$ ). On quantitative immunoprecipitation, misfolded wtSOD1 was found to constitute ~5% of total SOD1 in spinal cord samples from SOD1 familial as well as sporadic ALS. SOD1 misfolding and toxicity can propagate within and between cells, prompting novel targeted therapies for all forms of ALS.

## OR-23: Elucidating the cellular mechanisms of prion propagation and clearance for devising new targets for intervention

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Prion diseases are infectious fatal neurodegenerative disorders of man and animals which are characterized by spongiform degeneration in the CNS. A hallmark is the accumulation of a misfolded and pathologic isoform (PrP<sup>Sc</sup>) of the host-encoded normal prion protein (PrP<sup>C</sup>). Understanding the precise mechanisms of prion conversion on a cellular level and how cells can react and counteract are crucial for devising drugs effectively targeting these diseases. We have extensively characterized intracellular aggregation, trafficking and degradation of prion proteins in prion-infected cells in recent years. Here, we focus on implications of two major cellular degradation pathways on prion replication and clearance. The first one is autophagy which we think can have a promoting and inhibiting role in prion infection. We have shown that prion clearance can be enhanced in vitro and in vivo by drug-induced activation of autophagy. We have analyzed chemical drugs known to induce autophagy and using siRNA approaches we demonstrated that induction of autophagy is the underlying mechanism for increased lysosomal clearance of prions. More recent work using cells compromised in autophagy has revealed that a certain level of autophagy is needed for establishing acute and persistent prion infection. We speculate that autophagy might represent a functional equivalent for a disaggregase function, which is postulated for seed fragmentation in prion propagation, similar to sonication in PMCA or Hsp104 in yeast prion biology. Taken together, there seems to be a fascinating interplay between prion infections and autophagy. The second pathway we are interested in is the proteasomal one. We challenged different cell lines by inducing ER-stress or inhibiting proteasomal activity and analyzed the subsequent repercussion on PrP metabolism, focusing strictly on PrP in the secretory pathway. Both events led to enhanced detection of PrP aggregates and significant increase of PrP<sup>Sc</sup> in persistently prion-infected cells, which could be reversed by overexpression of proteins of the

cellular quality control. Remarkably, upon proteasomal impairment, an increased fraction of misfolded, fully glycosylated PrP molecules traveled through the secretory pathway and reached the plasma membrane. These findings suggest a novel pathway which possibly provides additional substrate and template for prion formation when protein clearance by the proteasome is impaired. Overall, these studies add to the understanding of the molecular requirements for cellular prion propagation and point to mechanisms which also might play a role in prion de novo generation as relevant in sporadic prion diseases.

## OR-24: Monitoring amyloid formation and maturation in vitro and in vivo using LCO fluorescence

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During the latest decade it has become obvious that amyloidogenic proteins and peptides can adopt a number of different non-native conformations. The roles of these different conformations in the amyloid cascade and their respective contribution to disease, have been frequently discussed. Classic amyloid targeting probes like Thioflavine T and Congo Red have proven useful for detection of mature amyloid in vivo as well as amyloid-like structures in vitro. To meet a growing need for fluorescent probes targeting pre-amyloid states on the misfolding pathway, a new type of probe with the commune name luminescent conjugated oligothiophenes (LCOs) are being developed. This group of probes comprises a number of different molecules with different properties regarding size, color, side chain substitution and binding properties.

The aim of our study is to discriminate between different stages of amyloid formation in vitro and in vivo, using a selection of these probes. We have monitored in vitro amyloid formation of b<sup>2</sup> peptides and recombinant human and mouse prion protein. In addition we have used spectral imaging fluorescence microscopy (Spectraview®) in combination with atomic force microscopy (AFM) to evaluate the forming of different misfolded conformations. In addition we have used different mouse models of Alzheimer disease at various ages to evaluate the plaque formation in vivo and change of amyloid constitution in mice over time. Size exclusion chromatography, Fluorescence lifetime

imaging (FLIM) and 2-photon excitation microscopy has also been used as readouts. Mice infected with different prion strains have also been evaluated.

Using a combination of these techniques we are able to discriminate between early and late stages of in vitro fibrillation in our different assays. The knowledge, on molecular level, earned from in vitro experiments are correlated with findings in brain tissue from mouse models of these cerebral amyloid diseases ranging over different ages and strains.

## OR-25: Prnp and susceptibility to prion diseases—From resistance to spontaneous disease

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Prion diseases are fatal transmissible neurodegenerative disorders of both animals and humans that include scrapie, bovine spongiform encephalopathy (BSE) and Creutzfeldt-Jakob disease (CJD). According to the protein-only hypothesis, prions are principally or entirely composed of an abnormal isoform (PrP<sup>Sc</sup>) of the host-encoded cellular prion protein, PrP<sup>C</sup>. Although it has become clear that other genes play a role, *Prnp*, the gene encoding for PrP, represents the major genetic determinant of prion diseases.

*Prnp* is highly variable in humans and several animal species and numerous mutations and polymorphic sites have been described.

During the last years, a wealth of data has been produced that demonstrates the influence of *Prnp* variations in conditioning the susceptibility, the clinical and pathological phenotype of prion diseases, their pathogenesis as well as the selection and mutation of prion strains. All these subjects are of crucial importance in prion diseases understanding.

The role of the PrP gene in influencing the susceptibility, the length of the incubation period and the clinical course of prion diseases is known since long time. In sheep, the identification of *Prnp* variants resistant to classical scrapie led to the implementation of innovative control strategies based on breeding programs for genetic resistance. However, the recent recognition of an atypical form of scrapie, named Nor98, affecting *Prnp* variants alternative to classical scrapie, along with its possible spontaneous origin, represent a challenge for breeding programs. The emergence of new prion strains is often related with *Prnp* variations. It is well known that on interspecies transmission, where recipient and prion donor have different *Prnp* genes, strain mutation may occur. Moreover, it has been shown that prion strains are actually composed by a range of PrP<sup>Sc</sup> conformers, each one having its own preference of replication in a given PrP sequence. It is therefore conceivable that *Prnp* variations can drive the evolution of strains both on interspecies transmissions, and on transmission within polymorphic species. This seems to emerge also from experiments of cross-breed transmission of scrapie in sheep, a highly *Prnp* polymorphic species. As a consequence of



mutation and selection, new molecular PrP signatures and new disease phenotypes can emerge, representing a challenge for diagnosis. Finally, the observation of spontaneous prion diseases in transgenic mice overexpressing specific *Prnp* sequences sheds new light on the origin of genetic and sporadic prion diseases and opens to the study of *Prnp* regions and conditions promoting such phenomenon.

Improving knowledge on the impact of *Prnp* variations in influencing prion diseases is essential for their diagnosis, the comprehension of their origin and progression, as well as for the design of appropriate approaches for their prevention, control and therapy. At the same time, the identification of genetic determinants for prion diseases different from *Prnp* has become a remarkable field of investigation.

### **OR-26: Molecular assessment of ruminant susceptibility for various TSE sources**

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Bovine spongiform encephalopathy (BSE) can be efficiently transmitted to small-ruminants (sheep and goats) with certain prion protein (PrP) genotypes. Polymorphisms in PrP of both host and donor influence the transmissibility efficiency of transmissible spongiform encephalopathies (TSEs) in general. PrP sequence differences (polymorphism collections) largely encode observed species-barriers and modulate the conversion of prion proteins underlying TSE agent replication.

In this study we demonstrate that protein misfolding cyclic amplification (PMCA) using a variety of PrP sources, can be used to quantify species- and polymorphism-barriers at the molecular level. Conversion efficiencies correlated well with observed differences in susceptibility and transmissibility of natural or experimental ovine and bovine TSEs in vivo. We assessed these species- and polymorphism-barriers within and between ruminant species in vitro using a variety of experimentally generated cross-species BSE agents. These include the frequently studied ovine-BSE derived from sheep having the ARQ/ARQ PrP genotype, but also two unique BSE-derived variants; one isolate of BSE passage in VRQ/VRQ sheep; one ovine-BSE (derived from ARQ sheep) isolate that was back-passaged into cattle; In addition, several goat-derived BSE isolates were studied as well.

Single-step (non-serial) PMCA reproduced the quantitative measurement of earlier established molecular conversion profiles in vitro correlating with known in vivo transmissibility and susceptibility in ruminant species. The PrP molecular weight signatures of the various strains were reproduced indicating conservation of strain-specific biochemical differences. Both

ovine-BSE isolates demonstrated a species-transmission profile reflecting the efficient transmission of certain sheep scrapie isolates into sheep while retaining its efficient conversion of bovine species as demonstrated for cattle BSE. Strikingly, the ARQ-derived ovine BSE has a much higher potency to species-wide PrP conversion, compared with sheep scrapie and cattle BSE. All data support that ovine-derived BSE, and presumably goat-derived BSE discovered recently, displays a significant increase in virulence as reflected by increased conversion potency and the efficient species-wide conversion of prion protein variants. In addition to efficient conversion profiles some data are presented on the inability of TSEs to efficiently adapt to the so-called resistant ARR PrP. National programs to genetically control TSEs in small ruminants, which are in place for several years in sheep, and which is currently being investigated for goats, seems still the most sound option to efficiently control TSEs in small ruminants.

**Acknowledgments.** This work was possible thanks to the EU funded projects BSE in sheep (QLRT-2001-01309) and GoatBSE (FOOD-CT-2006-36353 [www.goatTSE.eu](http://www.goatTSE.eu)), and the Dutch Ministry of Economic Affairs, Agriculture and Innovation, projects WOT-01-002-001.01 and WOT-01-002-001.05.

### **OR-27 Comparative analysis of prion proteins for evolutionarily diverse vertebrate species, polymorphic variants and mutants—Structure and essential dynamics**

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**Introduction.** Transmissible spongiform encephalopathies (TSE) are fatal neurodegenerative diseases affecting animals and humans. TSEs are associated with the conversion of normal prion proteins (PrP<sup>c</sup>) into an abnormal infectious form known as PrP<sup>Sc</sup>. Numerous studies have shown that certain mutations in the prion protein can lead to either increased or reduced risk (resistance) for the development of TSEs. It is also known that not all animal species are susceptible to prion diseases. Indeed, differences in the PrP<sup>c</sup> amino acid sequences, and the corresponding molecular structures, no doubt contribute to the susceptibility differences. Should particular sequence and/or structural trends associated with TSE resistance be elucidated, this could help clarify the molecular mechanisms behind the conversion, allowing the prediction of TSE genetic risks based on PrP sequence data. This information could eventually facilitate identifying, cloning or breeding TSE resistant animals.

**Methods.** A total of 25 PrP<sup>c</sup> constructs have been analyzed using NMR and X-ray structures available in the PDB. These include WT prion proteins of TSE-prone species (elk, cow, cat, hamster, humans), resistant species (frog, turtle, chicken, rabbit), sheep variants (ARQ, ARH, VRQ, ARR), and human mutants (E200K, V210I, Q212P). Molecular dynamics (MD) simulations

were performed for these constructs and the results were analyzed by essential collective dynamics (ECD).<sup>1</sup> ECD is a novel approach that allows one to identify, with atomic resolution, persistent dynamic correlations based on relatively short MD trajectories. The method has been validated by comparison with NMR experiments, and proven to provide an accurate assessment of dynamic domains,<sup>1</sup> main-chain flexibility,<sup>2,3</sup> and side chain dynamics<sup>4</sup> in proteins.

**Results and Conclusions.** Our ECD analysis indicates that the main chain in the area of helix HC is the most rigid and stable part in all the PrP constructs, whereas the highest level of dynamic disorder occurs in the area of the S1-HA loop. In many cases, the  $\beta$ -strands S1 and S2 are decoupled dynamically from each other indicating a scarcity of strong interactions between them. These trends appear to be pertinent to PrPs of both TSE-prone and resistant species. However, systematic differences among the species were found in the main-chain dynamics around the S2-HB loop. Prion proteins of TSE-resistant species (frog, turtle, chicken, rabbit) tend to have a relatively flexible S2-HB loop. Unlike the evolutionarily different species, point mutations in ovPrP and huPrP constructs have not been found to affect the main-chain dynamics. In contrast, these mutations did influence the dynamics of side chains, both around the mutation sites and at distant locations. In particular, the protective mutation H171R in ovPrP appears to have a stabilizing effect on the side-chain dynamics throughout the molecule, except for a number of residues in the S2-HB loop area. If these trends are validated by our ongoing numerical tests and NMR experiments, one can expect that the prion conversion risks could be predicted based on the amino acid sequences of PrP<sup>C</sup> constructs employing the ECD methodology.

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### OR-28: The cellular prion protein mediates neurotoxic signaling of scrapie prions and A $\beta$

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Neuronal dysfunction in different neurodegenerative diseases, such as Alzheimer disease, Parkinson disease, polyglutamine diseases or prion diseases is causally linked to aberrant protein folding. Our goal is to reach an understanding of how neurotoxic conformers are formed within the context of neuronal cells and

interfere with neuronal integrity. We have previously established a cell culture model to shown that PrP<sup>C</sup>-induced cell death is dependent on the expression of GPI-anchored PrP<sup>C</sup> and to identify domains of PrP<sup>C</sup> required for this activity.<sup>1</sup> Employing cell lines and primary neurons prepared from wild-type or PrP<sup>0/0</sup> mice we recently showed that PrP<sup>C</sup> can interact with and mediate toxic signaling of various  $\beta$ -sheet-rich conformers of different origins, including A $\beta$ , suggesting a pathophysiological role of the prion protein beyond prion diseases. Notably, the N-terminal domain of PrP<sup>C</sup> mediated binding to the  $\beta$ -conformers, and a secreted version of the isolated N-terminal domain interfered with toxic signaling via PrP<sup>C</sup>.<sup>2</sup> The mechanisms underlying the toxic activity of  $\beta$ -sheet-rich conformers are far from being understood, but it is conceivable that different upstream events converge at similar neurotoxic signaling pathways. We therefore started to define components downstream of PrP<sup>C</sup> involved in toxic signaling. Interestingly, A $\beta$ -, PrP<sup>Sc</sup>- and  $\beta$ -peptide-induced toxicity was significantly reduced by pharmacological blockage of NMDA receptor activity. In addition, we observed that PrP<sup>Sc</sup> can induce mitochondrial alterations in neurons, such as perinuclear clustering of mitochondria.

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### OR-29: The role of PrP in synaptic function and repair—Implications for treatment of prion and Alzheimer diseases

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The cellular prion protein, PrP<sup>C</sup> has been implicated in numerous cellular functions including signaling, neurite growth, neurogenesis, and neuroprotection.<sup>1,2</sup> PrP<sup>C</sup> is converted to its misfolded conformer, PrP<sup>Sc</sup>, in prion disease, associated with synaptic loss and dysfunction, cognitive deficits, and neuronal death. Early synaptic and cognitive changes can be reversed by neuronal depletion of PrP<sup>C</sup> in mice with prion disease, thus preventing conversion to PrP<sup>Sc</sup>, resulting in rescue of neuronal loss and long-term survival of the mice.<sup>3-5</sup> However, the toxic species and neurodegenerative mechanisms in prion disease are still largely unknown. Recent evidence has also implicated PrP<sup>C</sup> in mediating the toxicity of Amyloid-beta1-42 (A $\beta$ ) oligomers in Alzheimer disease (AD).<sup>6</sup> The interaction between A $\beta$  and PrP<sup>C</sup> has been shown to mediate the toxic effects of A $\beta$  on LTP and synaptic function in vitro, and on certain tests of memory in vivo.<sup>6-8</sup> However, conflicting reports exist, where the effects of A $\beta$  have been found to

be independent of PrP.<sup>7,9</sup> If PrP does indeed mediate A $\beta$  toxicity, it becomes an attractive target for therapy not only in prion diseases, but for AD as well. While PrP knockout is well tolerated in terms of survival, a better understanding for PrP's physiological function is important for potential future therapies for both AD and prion diseases.

In this study we have examined the role of PrP<sup>C</sup> in synaptic function and structural plasticity. First, we tested the effects of loss of function of PrP in early prion disease. As human PrP<sup>C</sup> is not converted to the scrapie form by mouse PrP<sup>Sc</sup> and would be expected to retain its function, we injected a lentivirus expressing human PrP<sup>C</sup> (huPrP) into the hippocampi of RML prion-infected mice, expressing mouse PrP<sup>C</sup>. We found that co-expression of huPrP prevented early synapse loss and synaptic dysfunction in prion diseased mice. These data suggest that early synaptic dysfunction in prion disease is due to the loss of PrP<sup>C</sup> function and its effects on synaptic number and function, while other mechanisms are involved in eventual neuronal death<sup>10</sup>.

We also examined the role of PrP in structural synaptic plasticity effects mediated by A $\beta$  oligomers. We injected synthetic A $\beta$  oligomers<sup>6</sup> into the hippocampus of both wildtype (WT) and PrP<sup>0/0</sup> mice. In both cases we found a significant reduction in spine density in the hippocampus 24 h after A $\beta$  oligomer injection, independent of PrP expression. Interestingly, recovery of spine loss was seen after 10 d in WT mice, but not in PrP<sup>0/0</sup> mice, nor in mice acutely depleted of PrP by RNAi. Further, we found that recovery of spine loss after A $\beta$  administration was rescued in PrP<sup>0/0</sup> mice by co-expressing mouse PrP in the hippocampus.

This study gives new insights into the possible role of PrP in synaptic function and structural plasticity and repair. Loss of PrP<sup>C</sup> function prevents synapse and spine recovery in early prion disease and after A $\beta$  mediated spine loss. Therefore, future PrP-targeting strategies for the treatment of prion disease and AD may have complex effects, both protective of direct toxicity, but inhibitory of synaptic repair processes.

## OR-30: Properties of geometry-locked PrPs and Shadoo protein in health and disease

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Prion diseases exhibit an accumulation of PrP<sup>Sc</sup>, a misfolded form of the C-terminal region of the host-encoded PrP<sup>C</sup> protein, while at the same time exhibiting a decrease in steady-state levels of the GPI-linked Shadoo (Sho) protein. In studies presented here we describe the properties of the Sho protein deduced from the behavior of Sho knockout and transgenic (Tg) mice. In a further series of studies we present the results of directed mutagenesis of the PrP N-terminal octarepeat region (residues 50–91), a region that has no exact equivalent in Sho, being instead represented by tandem RGG repeats N-terminal to the hydrophobic domain. Spectroscopic studies of Cu(II) titrated against PrP N-terminal fragments demonstrate three different metal coordination geometries denoted as “components 1, 2 or 3”<sup>1</sup> that bind 4, 2 or 1 copper ion(s) per octarepeat region, respectively. By reiterative peptide design, octarepeat region variants were produced with discrete component 1 (S1 allele) or component 3 (S3 allele) copper-binding geometries. The sequences encoding the variant octarepeat peptides were then engineered into a full-length mouse PrP coding region and used to produce Tg mice. CNS expression of component 3-locked PrP<sup>C</sup> (S3-PrP<sup>C</sup>) produced an abundant C-terminal fragment similar to “C2 PrP,” while component 1-locked PrP<sup>C</sup> (S1-PrP<sup>C</sup>) behaved in a similar manner to wt PrP. Transgenes encoding PrP with alternative “locked” Cu(II)-binding geometries exhibit novel properties during the course of prion infections thus illustrating a surprising effect of N-terminal sequences on the properties of the C-terminal protease-resistant core

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### OR-31: Prion strains in human prion diseases— Variably protease-sensitive prionopathy compared with other sporadic prion diseases

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The issue of prion strains in sporadic prion diseases has recently become more complex mostly because of three events: (1) the discovery of variably protease-sensitive prionopathy (VPSPr), a new, “sporadic” prion disease that, as sporadic Creutzfeldt-Jakob disease (sCJD), affects all three genotypes determined by the methionine/valine (M/V) polymorphism at codon 129 of the prion protein (PrP) gene; (2) the realization that distinct disease phenotypes are associated with the same genotypes-scrapie PrP (PrP<sup>Sc</sup>) type combination such as sCJDMM2 and sFI as well as sCJDMV2; and (3) the observation that a significant number of sCJD cases are associated with both types 1 and 2 of PrP<sup>Sc</sup>, the two basic species of PrP<sup>Sc</sup> in human sporadic prion diseases.

In VPSPr, PK-resistant PrP<sup>Sc</sup> shows very distinct features. (1) It forms five co-migrating electrophoretic bands spanning 26kDa to 7kDa in all 129 subtypes that are replicable by animal transmission and PMCA. (2) It lacks the diglycosylated isoform. (3) It includes internal and C-terminal fragments as well as large anchorless fragments; it differs in PK-resistance and the antibody immunoreactivity among the three 129 subtypes. (4) It may include a minor sCJD-like PrP<sup>Sc</sup> type 1 component in central brain nuclei. Furthermore, the prevalence of the three 129 disease subtypes in VPSPr (12% MM; 26% MV; 62% VV) is almost the opposite of that of sCJD (70% MM; 11% MV; 19% VV) indicating that the 129 genotype plays a different role as risk factor.

The insoluble PrP<sup>Sc</sup> preparations from sCJDMM2 and sporadic fatal insomnia (sFI) show distinct profiles in 1D- and 2D electrophoresis pointing to differences in glycosylation in insoluble PrP<sup>Sc</sup> present in these two conditions. PK-resistant PrP<sup>Sc</sup> also reveals distinct profiles in sCJDMV2 depending on whether the phenotype is “classical” with kuru plaques or sCJDMM2-like. Ongoing studies should characterize further these PrP<sup>Sc</sup> differences.

The co-occurrence of PrP<sup>Sc</sup> type 1 and 2 in all three 129 subtypes of sCJD is unquestionable although only one type can be detected in a significant number of cases. However, the PrP<sup>Sc</sup> two-type co-occurrence does not generate a third phenotype distinct from those associated with the types 1 and 2 occurring alone but rather a hybrid of type 1 and 2 phenotypes. Of notice, when PrP<sup>Sc</sup> types 1 and 2 co-occur in the same brain area and in comparable amount, type 1 seems to adopt the conformational characteristics of PrP<sup>Sc</sup> type 2, possibly generating a new strain.

In conclusion, VPSPr has introduced a novel and very different prion strain to sporadic human prion diseases which may have similarities with those associated with GSS, an exclusively inherited disease. However it may include small amounts of PrP<sup>Sc</sup> type 1.

Distinct prion strains are associated with different phenotypes that share 129 genotypes and PrP<sup>Sc</sup> type indicating that strain determinants other than the 129 genotype play a role.

Co-occurrence of PrP<sup>Sc</sup> types 1 and 2 in sCJDMM and sCJDVV does not create new phenotypes but one of the two co-occurring PrP<sup>Sc</sup> types may template conformational characteristics on the other.

**Acknowledgments.** Supported by NIH Award AG-14359, CDC Award UR8/CCU515004 and the Charles S. Britton Fund.

### OR-32: Human prion diseases in The Netherlands (1998–2009)—Clinical, genetic and molecular aspects

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Patients suspicious for a human prion disease in the Netherlands must be reported in a national surveillance program with 70.5% autopsy rate. Based on this program we describe the clinical, neuropathological, genetic and molecular characteristics of 162 autopsied patients with confirmed prion disease over a 12-y period (1998–2009), according to the Parchi classification 2009.<sup>1</sup> Since 1998, there has been a relatively stable mortality of Creutzfeldt-Jakob disease (CJD) in the Netherlands, ranging from 0.63 to 1.53 per million inhabitants per annum. Genetic analysis of the codon 129 methionine/valine (M/V) polymorphism in all patients with sporadic CJD (sCJD) showed a trend for under-representation of VV cases (7.0%), compared with sCJD cohorts in other Western countries, whereas the MV + 2K genotype was relatively over-represented (22.4%). Combined PrP<sup>Sc</sup> and histopathological typing identified all sCJD subtypes known to date, except for the VV1 subtype. In particular, a “pure” phenotype was demonstrated in 60.1% of patients, whereas a mixed phenotype was detected in 39.9% of all sCJD cases. The relative excess of MV cases was largely accounted for by a relatively high incidence of the MV 2K subtype. Genetic analysis of the prion protein gene (*PRNP*) was performed in 161 patients and showed a mutation in 9 of them (5.6%), including one FFI and four GSS cases. Several new mutations were found including Y226X and Q227X mutations, leading to anchorless prion accumulation with respectively CAA



and amyloid plaques and tangles<sup>2</sup>. Iatrogenic CJD was a rare phenomenon (3.1%), mainly associated with dura mater grafts. CJD after injection with infected pituitary gland hormones at early age was seen twice with incubation times of maximum of 28 y. Three patients were diagnosed with new variant CJD (1.9%) and one with variably protease-sensitive prionopathy (VPSPr). Post-mortem examination revealed an alternative diagnosis in 156 patients, most commonly Alzheimer disease (21.2%) and/or vascular causes of dementia (19.9%). The mortality rates of sCJD in the Netherlands are similar to those in other European countries, whereas iatrogenic and genetic cases are relatively rare. The unusual incidence of the VV2 sCJD subtypes compared with that reported to date in other Western countries deserves further investigation.

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## OR-33: Thalamic degeneration clinically presenting as CJD—More frequent than assumed?

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The thalamic form of Creutzfeldt-Jakob disease (CJD) (also known as sporadic fatal insomnia) is a rare phenotypic subtype of sporadic human prion disease. No such cases have been encountered among the 205 definite CJD cases recognized by the Austrian Surveillance during the period 1993–2010. In the present study we performed a comprehensive clinicopathological, biochemical and genetic study on four patients whom we have observed during 2011. Clinical symptoms included progressive dementia and ataxia in all four patients, extrapyramidal symptoms (Parkinsonism or chorea) in three of them, while myoclonus, psychiatric symptoms were noted in two. Additionally, pyramidal symptoms or prominent autonomic disturbance were mentioned in single patients. CSF 14-3-3 was assayed and was positive in two patients. The duration of illness was 7, 9, 12 and 12 mo, and age at death 74, 62, 55, and 77 y, respectively. Neuropathological examination revealed symmetrical thalamic degeneration characterized by prominent neuronal loss and gliosis in the dorsomedial and intralaminar and less in the anterior and lateral nuclei. This was associated with gliosis and pseudohypertrophy of the inferior olivary nucleus and focal neuronal loss and astroglia in the neocortex. The hippocampus, basal ganglia, and cerebellum did not show prominent alterations, and

we did not observe obvious spongiform change in any regions. Immunostaining for disease-associated PrP revealed scattered granular deposits in a synaptic and periaxonal distribution in a few neocortical regions. These tiny deposits were not observed in paraffin-embedded tissue blots. Immunostaining for  $\beta$ -amyloid, phospho-tau,  $\alpha$ -synuclein, TDP-43, FUS, ubiquitin, and p62 did not show any type of intracellular inclusions. PrP<sup>res</sup> was undetectable by western blotting in frontal cortex and cerebellum from three of these cases (two MM and one MV at codon 129), using a standard method or when the sample was first enriched for PrP<sup>res</sup> by centrifugation or by phosphotungstic acid precipitation. In summary, (1) these cases could be clinically classified as probable (two) and possible (two) CJD; (2) neuropathologically they were characterized by prominent symmetrical thalamic degeneration with subtle neocortical disease-associated PrP deposits; and (3) immunoblotting did not confirm the presence of PrP<sup>res</sup> in a restricted sample set tested. These cases present a challenge for current diagnostics, including the need for better characterization of the abnormal PrP conformers present in such cases, and emphasize once more the need for strict neuropathological survey in patients with rapidly progressive dementia. Such cases might be more frequent than previously assumed, given that we observed four such cases within a 12 mo period during which only 12 other cases of CJD were identified in Austria.

**Acknowledgments.** This study was performed in the frame of the Austrian Surveillance for Human Prion Diseases (OERPE) supported by the Federal Ministry of Health, Austria.

## OR-34: An update of transfusion transmission of variant Creutzfeldt-Jakob disease (vCJD)

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There have been 4 vCJD infections linked to blood transfusion in the UK, but there are a small number of individuals who remain clinically unaffected, despite being exposed to a blood transfusion derived from an individual who later developed vCJD. There are number of variables that may influence the risk of transfusion transmission and these include the time elapsed since the transfusion, the timing in relation to clinical onset of symptoms in the donor, the influence of leuco-depletion and the genetic background of recipients. Mathematical models suggest that there are likely to be further cases of transfusion transmitted vCJD in the future and that these cases may occur over an extended time frame. Concerns regarding the potential for transmission of vCJD through plasma products have been heightened by the identification of abnormal prion protein in the spleen of a patient with hemophilia, but there is a potential disparity between estimates of the number of individuals potentially exposed to significant infection and the absence of observed cases of clinical vCJD in exposed populations.

### OR-35: Efficacy of ethanol precipitation (TI+III+TII) and nanofiltration in removal of a high speed purified non sedimentable prion spike

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The validation of prion removal steps in the manufacturing of medicinal plasma-derived products is performed in reduced scale settings with prion spikes derived from brains of experimentally infected animals that exhibit high infectious titers. The nature of the spike used in these experiments is a matter of debate in terms of relevance to the real situation of blood infectivity. We have investigated the behavior of the non sedimentable prion infectivity (called SHS) obtained by Berardi et al. (2006)<sup>1</sup> from 263K scrapie brains, through two major biological safety methods that have proven their prion removal efficacy on a number of occasions: (1) ethanol fractionation involving precipitation and depth filtration in the manufacture of IgGs from TI+III +TII; and (2) nanofiltration through a sequence of filters of decreasing porosity (from 100 to 15 nm) from different manufacturers using albumin as a model protein. In both studies, we measured the infectious titers in spiked loads as well as in intermediate and in final products by animal bioassays. Our experiments showed that both ethanol fractionation and sequential nanofiltrations significantly removed prion infectivity contained in the SHS preparation, assumed to contain either “soluble” forms of prions or forms associated with lipids and which could constitute a valid model of poorly aggregated forms of prions. As already shown with other spike preparations, infectivity is recovered in the discarded precipitate I+III from the ethanol fractionation experiment. In the nanofiltration study, a 99.8% cumulative removal of infectivity was achieved with the 15-nm filter.<sup>2</sup> The results of the two studies will be presented and discussed in the context of spike relevance for prion validation studies.

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### OR-36: A new neurological disease in primates inoculated with prion-infected blood or blood components

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**Background.** Concerns about the blood-borne risk of prion infection have been confirmed by the occurrence in the UK of four transfusion-related infections of vCJD (variant Creutzfeldt-Jakob disease), and an apparently silent infection in an hemophilic patient. Asymptomatic incubation periods in prion diseases can extend over decades in humans, and a typical disease may or may not supervene. We present here unexpected results of independent experiments to evaluate blood transmission risk in a validated non-human primate model of prion disease.

**Methods.** Cynomolgus macaques were inoculated with brain or blood specimens from vCJD infected humans and vCJD or BSE-infected monkeys. Neuropathological and biochemical findings were obtained using current methods used for human patients.

**Findings.** Thirteen out of 20 primates exposed to human or macaque blood-derived components or potentially contaminated human plasma-derived Factor VIII exhibited an original neurological disease (myelopathy) previously not described either in humans or primates, and which is devoid of the classical clinical and lesional features of prion disease (front leg paresis in the absence of central involvement, lesions concentrated in anterior horns of lower cervical cord, with no spongiosis or inflammation), while the 12 brain-inoculated donor animals and one transfused animal exhibited the classical vCJD pattern. No abnormal prion protein (PrPres) was detected by standard tests in use for human prion diagnosis, but higher amounts of protease-sensitive PrP were detected in cervical cords than in controls. No alternative cause has been found in an exhaustive search for metabolic, endocrine, toxic, nutritional, vascular and infectious etiologies, including a search for pathogen genotypes (“deep sequencing”). Moreover, all the three animals transfused with blood treated with a prion removal filter remain asymptomatic with a one-third longer incubation period than the two animals transfused before filtration, which both developed the atypical syndrome presented here.

**Interpretation.** We describe a new neurological syndrome in monkeys exposed to various prion-infected inocula, including a potentially infected batch of plasma-derived Factor VIII. Our experimental observations in the absence of evident alternative etiology is highly suggestive of a prion origin for this myelopathy, that might be compared under some aspects to certain forms of

human lower motor neuron diseases. Similar human infections, were they to occur, would not be identified as a prion disease by current diagnostic investigations.

### **OR-37: Conditional cross-seeding of normal and vasculotropic mutant amyloid $\beta$ -protein in transgenic mice**

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**Introduction.** Intracerebral or peripheral delivery of exogenous amyloid  $\beta$ -protein ( $A\beta$ ) extracts prepared from brains of aged human  $A\beta$ PP transgenic mice have been reported to seed de novo amyloid formation in younger  $A\beta$ PP transgenic mice of the same strain. These findings suggest that pathogenic  $A\beta$  aggregates may exhibit prion-like propagating properties. Vasculotropic mutant  $A\beta$  leads to the preferential deposition of amyloid in cerebral blood vessels rather than in the brain parenchyma that is commonly observed with normal, non-mutated  $A\beta$ .

The aim of this study was to determine if normal and vasculotropic mutant  $A\beta$  could cross seed the deposition of amyloid in the brain parenchyma or in cerebral blood vessels.

**Material and Methods.** Brain extracts were prepared from aged Tg-5xFAD mice, a line that produces normal human  $A\beta$  peptides and develops early-onset, extensive parenchymal amyloid deposition and from aged Tg-SwDI mice, a line that produces vasculotropic mutant human  $A\beta$  and develops early-onset, extensive cerebral vascular amyloid. Amyloid-containing brain extracts from each strain were injected intracerebrally or peripherally into the opposite strain mice at three months and the mice were aged for an additional six months. We also bred the two strains of mice to generate bigenic Tg-5xFAD/Tg-SwDI mice producing both normal and vasculotropic mutant  $A\beta$  peptides together in brain and the mice were aged. Additionally, we bred Tg2576 mice, a line that produces normal human  $A\beta$  but develop later-onset parenchymal amyloid deposition. ELISA analysis was performed to measure cerebral  $A\beta$  levels and immunohistochemistry was performed to determine the spatial and quantitative accumulation of cerebral amyloid.

**Results.** Neither the intracerebral nor peripheral administration of amyloid-containing brain extracts from aged Tg-5xFAD mice or Tg-SwDI mice promoted the accumulation of de novo parenchymal or cerebral vascular amyloid in the same or opposite strain. However, in the bigenic Tg-5xFAD/Tg-SwDI mice where there is co-production of normal and vasculotropic mutant  $A\beta$  peptides in brain and early-onset parenchymal plaque formation there was higher accumulation of cerebral  $A\beta$  peptides, higher parenchymal amyloid plaque load, and loss of cerebral vascular amyloid. On the other hand, in bigenic Tg2576/Tg-SwDI mice where there is again co-production of normal and vasculotropic mutant  $A\beta$  peptides in brain but later onset parenchymal plaque

formation there was again a higher accumulation of cerebral  $A\beta$  peptides but in this case more severe cerebral vascular amyloid.

**Conclusion.** In these particular transgenic mouse lines the intracerebral or peripheral administration of amyloid containing brain extracts was not effective in promoting de novo cerebral amyloid formation. In bigenic Tg-5xFAD/Tg-SwDI mice early parenchymal plaque formation seeded higher amyloid plaque load whereas in bigenic Tg2576/Tg-SwDI mice early cerebral vascular amyloid formation seeded higher vascular amyloid load. Co-production of normal and vasculotropic mutant human  $A\beta$  in brain can promote higher amounts of cerebral amyloid formation although its location is dependent on the site of the earlier initial deposition.

### **OR-38: Genesis of mammalian prions**

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The transmissible agent of prion disease consists of a prion protein in its abnormal,  $\beta$ -sheet rich state ( $PrP^{Sc}$ ), which is capable of replicating itself according to the template-assisted mechanism. This mechanism postulates that the folding pattern of a newly recruited polypeptide chain accurately reproduces that of a  $PrP^{Sc}$  template.

Here we report that authentic  $PrP^{Sc}$  and transmissible prion disease can be generated de novo in wild type animals by recombinant  $PrP$  (rPrP) amyloid fibrils, which are structurally different from  $PrP^{Sc}$  and lack any detectable  $PrP^{Sc}$  particles.<sup>1</sup> When induced by rPrP fibrils, a long silent stage that involved two serial passages preceded development of the clinical disease. Once emerged, the prion disease was characterized by unique clinical, neuropathological, and biochemical features. The long silent stage to the disease was accompanied by significant transformation in neuropathological properties and biochemical features of the proteinase K-resistant  $PrP$  material ( $PrP^{Res}$ ). Specifically, accumulation of transmissible, partially PK-resistant  $PrP$  state that structurally resembled rPrP fibrils was observed during three serial passages before authentic  $PrP^{Sc}$  evolved.

The current work illustrates that transmissible prion diseases can be induced by  $PrP$  structures different from that of authentic  $PrP^{Sc}$ . These studies suggest that a new mechanism responsible for prion disease different from the classical  $PrP^{Sc}$ -templated conversion or spontaneous conversion of  $PrP^C$  into  $PrP^{Sc}$  exists. This new mechanism designated as “deformed templating” postulates that a change in the  $PrP$  folding pattern from the one present in rPrP fibrils to an alternative specific for  $PrP^{Sc}$  can occur. Consistent with this mechanism, previous work on molecular imaging demonstrated that significant switch in folding pattern can occur within individual fibrils or particles.<sup>2</sup> The current work provides important new insight into the mechanisms underlying genesis of the transmissible protein states and has numerous

implications for understanding the etiology of neurodegenerative diseases.

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### OR-39: Highly infectious recombinant prions—A new challenge for understanding how prions work

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A version of Protein Misfolding Cyclic Amplification (PMCA) based on recombinant PrP (rec-PrP) as substrate (rec-PMCA) has been used for generating highly resistant PrP<sup>res</sup>. rec-PrP from hamster, sheep, bank vole, mouse, pig, cow, human, and other species have been seeded with a diversity of prion strains in order to generate self-replicating proteins that display the principal characteristics attributed to mammalian prions. The fact that biochemically and biologically distinguishable rec-PrP<sup>Sc</sup> are being generated in vitro from the same species proves that the GPI and glycosylation components are not necessary in enciphering different strains and different conformations. Electronic microscopy images from all of these rec-PrP<sup>res</sup> are reminiscent of those observed in GPI-less PrP<sup>Sc</sup> preparations (rods) and clearly are distinguishable from those observed in standard fibril preparations. Infectivity is being tested in different wild type and transgenic mouse models, showing that while the infectivity is high (100% attack rate), new prion strains with atypical characteristics are emerging. rec-PMCA is also being used for estimating the transmission barrier in a more reliable and accurate way. Infectious rec-PrP<sup>Sc</sup> opens new avenues for structural studies. In particular, we have used rec-PMCA to generate enough material to characterize the PK resistant cores of different rPrP<sup>Sc</sup> strains using mass spectrometry. These studies confirm a basic structural similarity of rPrP<sup>Sc</sup> and brain-derived PrP<sup>Sc</sup>.

### OR-40: Prion-like acceleration of a synucleinopathy in a transgenic mouse model

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**Introduction.** The common feature of synucleinopathies, such as Parkinson disease or Lewy bodies dementia, is the accumulation of intracellular aggregates of  $\alpha$ -synuclein ( $\alpha$ -syn) that spread throughout the nervous system. A number of recent studies highlighted possible similarities with prions of propagation of the  $\alpha$ -syn pathology in humans affected with such diseases.<sup>1</sup> Our aim was to investigate experimentally the possible transmission in vivo of a synucleinopathy, using a transgenic mouse model (TgM83) overexpressing a mutated form (A53T) of the human  $\alpha$ -syn protein.<sup>2</sup>

**Material and Methods.** Brain homogenates from old TgM83 transgenic mice showing characteristic motor clinical signs of the synucleinopathy and containing insoluble and phosphorylated  $\alpha$ -syn were intra-cerebrally inoculated in young mice.

**Results.** This triggered, only in TgM83 inoculated mice, an early onset of motor clinical signs compared with uninoculated TgM83 mice. This early disease was associated with phosphorylated  $\alpha$ -syn as identified by both western blot and immunohistochemistry in old and symptomatic uninoculated TgM83 transgenic mice. Inoculation of brain homogenates from these mice also triggered acceleration of the disease after their inoculation in young TgM83 mice ("second passage"). The characteristic clinical signs are only observed in mice overexpressing the human A53T mutated  $\alpha$ -syn, but not in similarly inoculated wild-type mice or transgenic mice overexpressing the normal human  $\alpha$ -syn protein (TgD9 mouse line).

**Conclusions.** Although the involved molecular mechanisms remain to be determined, acceleration of the pathology following inoculation of TgM83 mice expressing mutated human  $\alpha$ -syn by brain tissues from TgM83 mice clinically affected by the synucleinopathy, could be consistent with a "prion-like" propagation of the disease. Similar results had been reported in transgenic mouse models expressing tau or  $\alpha$ -amyloid proteins involved in Alzheimer disease, but not yet in mice expressing the  $\alpha$ -syn protein.<sup>3,4</sup>

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## OR-41: Prions lacking glycosylated PrP at the first N-glycosylation site in both variably protease-sensitive prionopathy and a familial prion disease

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**Background.** The newly-identified sporadic prion disease termed variably protease-sensitive prionopathy (VPSPr) is characterized by clinical and neuropathological features as well as the deposition in the brain of a scrapie prion protein (PrP<sup>Sc</sup>) that are different from those Creutzfeldt-Jakob disease (CJD).<sup>1,2</sup> The molecular mechanism underlying the formation of the VPSPr-associated, distinctive PrP (PrP<sup>Dis</sup>) remains unknown.

**Methods.** Brain tissues from VPSPr, sporadic CJD, and familial CJD<sup>V180I</sup> patients were obtained at autopsy and all collected at the National Prion Disease Pathology Surveillance Center, Cleveland, USA, except one fCJD<sup>V180I</sup> case from France. One- and two-dimensional Western blotting were used to detect PrP in frozen human tissues and cell lysates. Histology and immunohistochemistry were used to examine fixed brain tissues. Human neuroblastoma cells (M17) transfected with human wild-type PrP (PrP<sup>Wt</sup>), PrP<sup>V180I</sup>, and PrP<sup>T183A</sup> coupled with either 129M or 129V polymorphism were used as cell models to investigate the effect of mutations on glycosylation and protease-resistance of PrP3. Anti-PrP antibodies included 3F4, 1E4, V14<sup>3,4</sup> and V61<sup>3,4</sup> were used.

**Results.** A naturally-occurring pathogenic mutation, valine (V) to isoleucine (I), at PrP residue 180 linked to familial Creutzfeldt-Jakob disease (fCJD<sup>V180I</sup>) exhibits a proteinase K (PK)-resistant PrP<sup>res</sup> that is strikingly similar to that observed

in VPSPr even though they are associated with either wild-type (PrP<sup>Wt</sup>) or mutated PrP, respectively. Lack of the diglycosylated PrP<sup>res</sup> species observed in both fCJD<sup>V180I</sup> and VPSPr is attributable to loss of PrP<sup>Dis</sup> glycosylated at the first N-linked glycosylation site at residue 181 (PrP<sup>Dis-181</sup>). Neither PrP<sup>Dis-181</sup> nor PK-sensitive PrP<sup>Dis</sup> glycosylated at residue 181 are detected in PrP captured from VPSPr and fCJD<sup>V180I</sup> by gene 5 protein and sodium phosphotungstate, reagents that have been proven to specifically capture both PK-resistant and -sensitive PrP<sup>Sc</sup>. Remarkably, the peculiar PrP<sup>Dis</sup> with the five-step ladder-like electrophoretic profile, a molecular hallmark of VPSPr, also appears in fCJD<sup>V180I</sup>. Unlike fCJD<sup>T183A</sup>, in which the mutation completely abolishes the first N-linked glycosylation site, both VPSPr and fCJD<sup>V180I</sup> show lack of diglycosylated PrP only in PK-treated but not in untreated PrP. In cultured cells, the diglycosylated PrP is detected in PrP<sup>Wt</sup> and PrP<sup>V180I</sup>, but not in PrP<sup>T183A</sup>.

**Provisional conclusion.** Our study suggests that fCJD<sup>V180I</sup> and VPSPr may share a unique pathogenetic mechanism involving an altered glycoform at the first N-linked glycosylation site that affects PrP conversion. Moreover, the altered glycoform occurred in VPSPr without PrP mutations may imply that one or more non-PrP factors may be involved in the pathogenesis of VPSPr.

**Acknowledgments.** Supported by the National Institutes of Health (NIH) NS062787, NIH AG-08012, AG-14359, the CJD Foundation, Alliance BioSecure, the University Center on Aging and Health with the support of the McGregor Foundation and the President's Discretionary Fund (CWRU) as well as CDC Contract UR8/CCU515004.

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