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Prion 2024 Conference Abstracts

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1. High resolution structures of brain-derived prion strains

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Aims: To determine the structures of infectious brain-derived prions to understand the molecular foundations of prion propagation and strain variation
Materials and Methods: Prion fibril purification from brain (mouse and deer); bioassays of purified fibrils; real time quaking induced conversion (RT-QuIC) assays; negative stain transmission electron microscopy (EM); cryo-electron tomography; cryo-EM; single-particle analysis; and 3D image reconstruction.
Results: SDS- PAGE and immunoblots of purified preparations showed high purity with respect to PrP content. Negative stain EM showed predominantly fibrillar morphology with mixtures of isolated, laterally associated, and crossed fibrils. Cryo- electron tomography showed that 263k, aRML and a22L rodent prion fibrils had left-handed twists. We also observed globules of unknown composition along the sides of some of the fibrils. Structural details of prion fibrils were obtained by single particle acquisitions and helical reconstruction.

So far, all brain-derived prion fibrils parallel in-register intermolecular β-sheets (PIRIBS)-based architectures. The rodent prions have a single PrP monomer spanning the fibril cross-section with N-, middle, and disulphide arch motifs and a steric zipper near the N-terminus of the ordered core. However, conformational differences within these motifs arches and other regions distinguish these strains, including the a22L and aRML prion strains isolated from the same genotype of mouse.

We currently have slightly lower-resolution data on a CWD prion. Clearly, it has a PIRIBS architecture and both similarities and differences to the known rodent prion strains. However, like the rodent scrapie strains, the CWD core structure is much larger than that of human GSS F198S prion fibrils.
Conclusions: The prion strains that have solved to date each have unique conformational templates on the fibril ends that dictates the fold of incoming PrP monomers as the prions grow, even when isolated from hosts of the same genotype. Our findings are providing structural insights into the molecular bases of prion strain propagation and transmission barriers. Precise knowledge of prion structures should also help in drug discovery and the design vaccine against prion diseases of animals and humans.
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Acknowledgement: We thank Ms. Elizabeth Fisher for helpful suggestions and oversight of the NIH EM facility.

2. Syntaxin-6 Modifies Prion Pathogenesis in vivo & in Cellular Models

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Aims: Syntaxin-6 (STX6), an intracellular trafficking protein, has been proposed as a risk gene for sporadic Creutzfeldt-Jakob disease (sCJD), with increased STX6 expression in the brain driving disease risk. However, little is known about the stage of prion disease pathogen-

esis syntaxin-6 is acting or the mechanism through which it exerts its risk effects. At Prion 2023, we presented the results of a prion transmission study assessing the effect of syntaxin-6 knockout on the two-phase kinetics model of prion replication proposed by the MRC Prion Unit. This suggested syntaxin-6 was not involved in prion propagation nor prion-induced neurotoxicity in vivo. The aim of this work was to assess the effect of syntaxin-6 manipulation on the establishment of prion infection in vivo, as well as explore cellular mechanisms.

Materials and Methods: Stx6-/- and Stx6+/+ mice were intracerebrally inoculated with RML prions at limited dilutions with mice culled following definite scrapie sick diagnosis, with validation by histological methods (deposits of abnormal PrP and vacuolation on H&E). Incubation periods and attack rates were calculated, with the 'effective dose' estimated by the Spearman-Karber method. Syntaxin-6 levels were manipulated in PK1 and CAD5 cells and prion propagation assessed using the scrapie cell assay (SCA) and changes in distribution of disease-related PrP assessed by confocal microscopy.

Results: When infected with limiting dilutions of RML, syntaxin-6 knockout in vivo reduces the effective dose by 1 log compared to wild-type controls. In fact, at dilutions 10–6 and lower, Stx6+/+ mice have approximately 3 times the odds of developing clinical scrapie disease compared to Stx6-/- mice. In chronically infected cells, knockdown of Stx6 leads to a redistribution of disease-related PrP (PrPd), with an increase of intracellular, perinuclear PrPd. Furthermore, prion propagation in PK1 cells was consistently altered across multiple different paradigms with Stx6 knockdown resulting in a robust increase in the spot count in the SCA with the converse observed with syntaxin-6 overexpression. Congruent findings were found with syntaxin-6 knockdown in CAD5 cells with there being evidence of increased prion propagation following infection with RML, 22 L, MRC2 and ME7 strains.

Conclusions: This work shows that syntaxin-6 knockout in vivo reduces susceptibility to infection suggesting its involvement in the initial establishment of prions, consistent with its risk discovery in human. Syntaxin-6 manipulation in cellular models alters prion-related phenotypes, in keeping with an intracellular trafficking phenomenon. These studies therefore firmly establish syntaxin-6 as a modifier of prion disease, informing on pathological mechanisms.

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Acknowledgement: All the staff at our biological services facility. Fabio Argentina, Tamsin Nazari and Jordin Orellana for help with histology.

3. Novel emergent CWD strains with unstable properties cause chronic wasting disease (CWD) in Nordic cervids

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Aims: Since 2016, CWD has been detected in increasing numbers of free-ranging reindeer (Rangifer tarandus), moose (Alces alces) and red deer (Cervus elaphus) from Norway, Finland, and Sweden. Our previous studies showed that strain differences between Nordic and North American CWD isolates can be identified using gene-targeted (Gt) mice which express physiologically accurate levels of elk or deer prion protein (PrP). Using this platform, we showed that Nordic CWD prions exhibit considerable strain diversity and instability. Here we assessed the transmission and phenotypic properties of prions causing CWD in Norwegian red deer, Swedish moose and Norwegian moose using Gt mice expressing different cervid PrP genotypes.

Materials and Methods: We intracerebrally inoculated the Gt mice with prions causing CWD in Norwegian red deer, Swedish moose and Norwegian moose. We collected brain, muscle and spleen from Gt mice, the biochemical properties of these prions were assessed using immunohistochemistry, western blotting, conformational analyses, and in vitro amplification platforms.

Results: Transmission of these non-lymphotropic Nordic CWD strains resulted in stochastic lymphotropic adaptation, and a unique neuropathological profile characterized by prion accumulation in the dentate gyrus region of the hippocampus.

Conclusions: Our results suggested that the adaptation from non-lymphotropic strains to lymphotropic strains may occur during the transmission of Nordic CWD prions. Our demonstration of novel emergent strains and their potential for evolution increases uncertainties about CWD zoonosis.

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4. Crucial factors contributing to the prolonged survival of patients with V180I genetic Creutzfeldt-Jakob disease

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Aims: Creutzfeldt-Jakob disease (CJD) with a point mutation of valine to isoleucine at codon 180 of the prion protein (PrP) gene is the most frequent genetic CJD (gCJD) form in Japan and is extremely rare in Europe and North America. Although cases of V180I gCJD generally involve longer survival periods and later disease onset compared to those with sporadic CJD (sCJD), details regarding the factors influencing survival are still unclear.
Materials and Methods: To determine the influence of certain factors on survival, we retrospectively assessed 19 Japanese patients with V180I gCJD with respect to background, clinical course, and disease management. Furthermore, we compared the obtained date with that of our previously reported 51 Japanese patients with MM1-type sCJD.
Results: In the former, no significant differences between survival periods were found between male (n = 3) and female (n = 16) patients and patients with methionine homozygosity (n = 15) and valine heterozygosity (n = 4) at polymorphic codon 129 of the PrP gene. The survival period of tube-fed patients (n = 11) was significantly longer than that of non-tube-fed patients (n = 8). Mechanical ventilation was not performed. Disease duration was significantly negatively associated with onset age, which was significantly later with V180I gCJD than with MM1-type sCJD. Total disease duration was significantly longer with V180I gCJD than with MM1-type sCJD.
Conclusions: V180I gCJD showed statistically longer survival periods and later disease onset than did sCJD. We concluded that the most crucial factor contributing to the prolonged survival of patients with V180I gCJD was tube feeding.
Funding: This work was supported by a grant-in-aid from the Research Committee of Prion Disease and Slow Virus Infection, the Ministry of Health, Labour and Welfare of Japan.

5. Comparative analysis of yeast prion strains formed by Sup35 protein derivatives with various deletions in prion domain

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Aims: Yeast prion protein Sup35 is a convenient model for studying amyloid aggregation that is distinctive hallmark of human neurodegenerative proteinopathies. Prion domain of Sup35 (Sup35N) includes N-terminal QN-rich stretch, octapeptide repeats and C-terminal region (CTN). QN stretch is critical for amyloid aggregation in yeast (Toyama BH et al. 2007 Nature 449: 233), while CTN is required in mammalian cells (Duernberger Y et al. 2018 Mol Cell Biol 38: e00111-18). We investigated the ability of proteins with deletions of either QN stretch or CTN region to maintain prion state in yeast cells and transfer it to each other.
Materials and Methods: Yeast genetic, biochemical and fluorescence microscopy techniques were employed.
Results: We demonstrated that the Sup35 derivatives lacking either QN or CTN region can maintain the prion state in yeast, however cannot cross-seed each other. QN deletion significantly impaired mitotic stability of prion and its dependence on the Hsp104 chaperone. Both prions formed by QN and CTN deletion derivatives were less sensitive to Hsp104 overproduction, compared to full-length Sup35 prions.
Conclusions: N-terminal and C-proximal amyloidogenic regions of Sup35N can maintain prion states separately from each other, but do not convert each other into a prion in trans configuration in yeast cells. Lack of either region influences interactions with the chaperone machinery to different extents.

6. Sup35N prion domain drives liquid-liquid phase separation during hyperosmotic shock in a pH-independent manner

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Aims: Cells employ compartmentalization to regulate biochemical reactions in living cells. Alongside with membrane-bound compartments (such as nucleus, mitochondrion, endoplasmic reticulum, etc.), membrane-less organelles (biomolecular condensates) are formed via liquid-liquid phase separation (LLPS) or gelation. LLPS is often driven by intermolecular interactions of proteins containing unstructured regions. For example, a yeast prion Sup35, translation termination factor (eRF3), forms both reversible biomolecular condensates and amyloid fibrils in Saccharomyces cerevisiae cells. Literature data indicated that Sup35 undergoes phase separation in response to the drop of cytosolic pH drop during starvation and acidification of medium (Franzmann T et al. (2018) Science 359(6371): eaao5654). The middle region of Sup35 (Sup35M) containing charged residues was implicated as a modulator of this process. We observed that hyperosmotic stress also leads to the formation of liquid condensates by Sup35 derivatives. This study addresses relationship between pH and osmotic stress in regard to Sup35 phase separation.
Materials and Methods: Sup35N derivatives fused to fluorescent proteins were overproduced in S. cerevisiae cells, lacking pre-existing prions. Formation of liquid biomolecular condensates by phase separation was monitored by fluorescence microscopy. Cytosolic pH was measured using pH-sensitive fluorescent protein sfpHluorin.
Results:Hyperosmotic shock slightly decreases cytosolic pH, but it remains near or above neutral. Sup35N region (prion domain) is required and sufficient for the phase separation promoted by hyperosmotic stress.
Conclusions:Our data shows that hyperosmotic stress promotes phase separation of Sup35 protein by the mechanism that is distinct from acidification of cytosol.
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7. Structural studies of de novo formation of [PSI+]

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Aims: [PSI+] is one of the best-studied yeast prions, formed by the aggregation of the translation termination factor Sup35p. Structural studies on Sup35p have demonstrated that the in vitro assembled fibrils have an in-register parallel β-sheet structure, but the structure of Sup35p fibrils from yeast cells remains undetermined. Overproduction of Sup35 prion-containing domain (Sup35NM) can dramatically increase the frequency of de novo induction of [PSI+]. With the fusion of GFP or YFP, the expression of [PSI+] could be detected by fluorescence microscopy. During the induction, a 'transition state' is characterized by the appearance of rings (or ribbons) assemblies, which consist of continuous bundles of long Sup35NM fibrils. Then they mature into an infectious prion state, with a single dot-shaped structure containing fragmented fibrils.

We will develop our protocol to stably purify Sup35NM fibrils from yeast cells containing mainly rings and dots only, respectively, and study the Cryo-EM structures of Sup35NM fibrils.
Materials and Methods: The Rings and Dots cells with a stably integrated construct consisting of Sup35NM-YFP under the control of Gal1 promoter, were grown in standard YPD media and induced in media containing 2% D-galactose (YPGal). The formation of rings-like and dots-like [PSI+] in yeast cells is checked under the confocal microscope. Ex vivo extracted Sup35NM fibrils are characterized by Negative-EM and confirmed by semi-denaturing agarose gels and immunogold labelling. We will use Cryo-EM to study the atomic structures of Sup35NM fibrils from rings and dots cells, respectively.
Results: Our optimized protocols for ex vivo extraction of [PSI+] allowed us to purify de novo formed Sup35NM fibrils. Using negative-stain EM, we observed long fibrils from rings cell while much shorter fibrils from dots cell. These fibrils were detected to be labelled by anti-Sup35 antibody via immunogold-labelling EM. When analysed on semi-denaturing agarose gel, the Sup35NM protein migrated as high-molecular-weight SDS-resistant complexes, typical of prion amyloids.
Conclusions: We have been able to stably purify ex vivo [PSI+] fibrils from yeast cells. The Sup35NM fibrils from Rings and Dots cells showed different lengths, consistent with published Cryo-ET data. The samples of good purity and high concentration can now be used for Cryo-EM structural study.
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8. Cryo-correlative imaging of prion strains ex situ

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Aims: Various in vitro models of prion propagation have produced important insights into prion infection, but there is limited knowledge about the structures of prion or PrP assemblies propagated in cells and the native context in which they form. We sought to implement cryo-correlative light, electron and X-ray tomography (cryo-CLEXT) to generate high-resolution near-native 3D reconstructions of prion-infected cells.
Materials and Methods: Primary mouse neurons and/or glia grown on cryo-EM grids were inoculated with fluorophore-tagged ex vivo mouse prion fibrils. Nascent PrP assemblies were propagated for up to 3 weeks post-inoculation and differentially immunolabelled, facilitating selective targeting of both prion inocula and the ensuing PrP assemblies by cryo-CLEXT in vitrified (frozen-hydrated) cells.
Results: Fluorophore-tagged clusters of fibrils from RML and 22 L mouse prion strains (inocula) appear to be internalized and destined for degradation in neurons or glia. Elongated nascent PrP assemblies incrementally form in and/or on the cells over the course of infection in a strain-specific manner. Both the inocula and the resultant PrP aggregates are readily detected under cryogenic conditions, enabling high-resolution cryo-CLEXT data collection for a comprehensive view of their structure and interactions within cellular nano-environments.
Conclusions: We have produced a reliable method for cryo-CLEXT of prion-infected neurons and glia, which will provide novel structural and mechanistic insights into prion propagation and strain selection in highly relevant types of brain cells.
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9. Flow Cytometry-based Quantification of Prion Protein from Human Blood Enables Measurement of Target Engagement for Prion-lowering Therapeutics

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Aims: Prion disease is a rare, fatal neurodegenerative disorder caused by misfolding and aggregation of host encoded prion protein (PrP). Evidence from genetic models and antisense oligonucleotide (ASO) studies indicate that lowering PrP in the brain is therapeutically beneficial. We are working to develop a small molecule therapeutic to selectively block synthesis of new PrP, thus lowering total PrP in the brain. Measuring target engagement in higher species and humans in early clinical development is crucial to determine the therapeutically active dose of drug. Current PrP detection methods in cerebrospinal fluid (CSF) are invasive and impractical for frequent monitoring. We propose a viable method for routine clinical assessment of target engagement by measuring PrP on the surface of key cell populations in human blood.
Methods: We sourced both cryopreserved and fresh peripheral blood mononuclear cells (PBMCs) from human, non-human primate (NHP), rat and mouse. We stained these cells with a panel of antibodies against CD45, CD3, CD19, CD14, CD4 and CD8 to identify the key immune cell populations, as well as with several commercially available monoclonal anti-PrP antibodies that had been reported to cross-react with multiple species and analysed the results using flow cytometry.
Results: Our study shows that PrP is detectable by flow cytometry on T cells, B cells, and monocytes isolated from human whole blood with minimal processing. PrP levels can be robustly measured on both live and fixed cells using standard laboratory procedures. Ex vivo treatment of PBMCs from healthy donors with PrP-lowering small molecules resulted in dose-dependent reductions in prion expression across immune subtypes. Notably, PrP was not measurable on mouse or rat immune cells but was robustly expressed on PBMCs isolated from NHPs.

Conclusions: These findings suggest that PrP can be measured robustly on peripheral immune cells. This method may allow for more frequent and routine measurement of prion protein reduction in patients during clinical trials of prion-lowering small molecule therapeutics. In addition, combining PrP measurements from CSF with frequent blood sample assessments may help increase our understanding of the pharmacokinetic/pharmacodynamic (PK/PD) relationship of a therapeutic in the periphery and central nervous system.

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10 Oral Small Molecules for Reducing Prion Protein Levels: A New Therapeutic Strategy for Prion Disease

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Aims: Evidence from genetic models and antisense oligonucleotide (ASO) studies indicate that lowering prion protein (PrP) in the brain is therapeutically beneficial in prion disease. Molecular Gates are small molecules that selectively eliminate extracellular proteins at their source. Molecular Gates block the interaction between a target protein's unique signal peptide and the secretory translocon through which the protein must pass to reach the cell surface (Rehan et al., 2023). Blocked from export, the nascent target protein is degraded (Kang et al., 2006). As small molecules, Molecular Gates can be administered orally and access the whole brain via the bloodstream. We have synthesized and tested PrP-selective Molecular Gates with the aim of showing potent and complete Prion-lowering in cell culture and substantial target engagement in the brain of mice dosed with compound. Here we present data from one of these compounds, MG-813.
Methods: We endogenously tagged the PrP locus of N2A (mouse neuroblastoma) and U251-MG (human glioblastoma) cells with a HiBit reporter (Promega) to monitor the levels of PrP using luminescence. We treated these cells with MG-813 at various concentrations for 6 hours. PrP levels were confirmed by Western Blotting. We then administered MG-813 or vehicle to C57BL/6 mice once per day by oral gavage for 8 days. We collected colon and brain from these animals and quantified PrP levels using ELISA and IHC. Compound concentration in plasma and tissues was also determined using LC-MS.
Results: MG-813 potently eliminated endogenous PrP expression from both mouse and human neural cell lines. In vivo experiments demonstrated that once per day oral dosing of MG-813 at 100 mg/kg for 8 days decreased the PrP concentration in the brain of mice by 80% and in the colon by 96%, relative to vehicle-treated mice.
Conclusion: Here we demonstrate that significant lowering of PrP in the brain of mice is possible with an orally administered small molecule with a novel mechanism. PrP-lowering ASOs are now being tested in the clinic. While promising, ASOs have limited potency related to their uneven distribution to the deep brain. In addition, long-term treatment requires invasive and repeat intrathecal dosing by lumbar puncture. By contrast, our novel mechanism paves the way for the development of a pill to treat or prevent prion disease.

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11. Mortality surveillance of persons potentially exposed to chronic wasting disease

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Aims: Wisconsin Department of Health Services, Division of Public Health (WDHS) personnel maintain a database consisting of information collected from hunters who reported eating, or an intention to eat, venison from chronic wasting disease (CWD)-positive

cervids. These data, collected since 2003, allow for the evaluation of causes of mortality in potentially exposed persons.

Methods: The WDHS database contains the name, date of birth, when available, year of CWD-positive deer harvest, city and state of residence, method of processing, and when applicable, names of others with whom the venison was shared, for each potentially exposed individual. For select years, names of all hunters who harvested a deer testing positive for CWD are also available. All names in the database are cross-checked with reported cases of human prion disease in Wisconsin and cases in the National Prion Disease Pathology Surveillance Center (NPDPSC) diagnostic testing database. Persons with date of birth available are also cross-checked with prion disease decedents identified through restricted-use national multiple cause-of-death data via a data use agreement with the National Center for Health Statistics (NCHS).

Results: The database currently consists of 1880 records for hunt years between 2003–2023, with 873 unique individuals with accompanying date of birth. No matches were found among any persons in the database cross-checked with WDHS human prion disease surveillance data, NPDPSC data (September 2023 update), and NCHS data through 2021.

Conclusions: The findings of this ongoing review are reassuring; however, the number of persons cross-checked so far is likely only a small percentage of those potentially exposed to CWD in Wisconsin. In addition, many more years of vital status tracking are needed given an expected long incubation period should transmission to humans occur.

12. A PrP-targeting antibody with a dual effect induces rapid clustering, uptake and degradation while also stimulating proteolytic surface shedding of PrP

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Aims: Multiple biological effects have been described for diverse PrP-directed antibodies, ranging from neuroprotection (even qualifying for therapeutic administration) to induction of toxicity. We have shown that several antibodies, upon binding to cell surface PrP, lead to its increased proteolytic release ('shedding') by the metalloprotease ADAM10, whereas one IgG (POM2, having four repetitive epitopes in PrP`s flexible N-terminal tail) causes fast formation of large PrP: antibody clusters at the membrane, followed by their endocytosis and lysosomal degradation. We have now identified one particular antibody combining both aspects. This study aims at an in-depth characterization of its mode(s) of action and therapeutic potential in neurodegeneration.

Materials and Methods: Treatment effects of the antibody candidate were so far assessed biochemically in several human and mouse cell lines, murine primary cells and slice cultures. We performed qPCR and a battery of toxicity/viability assays to assess potential transcriptional and adverse effects, respectively. In addition, we undertook live, immuno electron and atomic force microscopy, as well as aggregation (e.g., LLPS assay) and structural studies (including small-angle X-ray scattering) to yield mechanistic insight.

Results: The 'bimodal' effects of the candidate to rapidly initiate formation, endocytosis and degradation of PrP: antibody complexes while also substantially stimulating the ADAM10-dependent shedding from the cell surface was confirmed in all models tested so far. We found no evidence for alterations in Prnp gene expression or for toxic effects caused by treatment with the candidate. Binding of the candidate to one PrP or crosslinking of two PrP molecules at the cell surface may trigger proteolytic shedding, whereas other binding modalities may enable fast formation of PrP: antibody complexes (likely at membrane subdomains with high PrP density, such as lipid rafts) that undergo uptake and degradation. Structural data is currently being analysed and will reveal further critical insight.

Conclusions: Reducing cellular PrP levels is considered neuroprotective with regard to neurodegenerative

proteinopathies and, hence, represents the goal of several recent therapeutic strategies. The antibody studied here combines this effect with an increased production of shed PrP, a released nearly full-length PrP fragment seemingly able to bind and sequester toxic protein/peptide assemblies in the extracellular space. This dual effect is unique among the several PrP-directed antibodies we have tested thus far. Functional assays in disease-relevant models will reveal whether it really holds treatment potential.

Funding: CJD Foundation, Inc. (USA); Alzheimer Forschung Initiative (AFI e.V., Germany); Chinese Scholarship Council (grant #202108080249)

13. Effect of copper and manganese on the persistence of chronic wasting disease prions

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Aims: Transitional metals, especially manganese and copper, can bind prion protein and were found to impact its properties. Previous studies reported mixed observations of the correlation between the metal level and prion disease progress. However, the effect of copper and manganese on the persistence of CWD prions in the environment are unclear. The aim of this study is to investigate the change of CWD prion property in a copper- or manganese-supplemented environment.

Materials and Methods: Natural elk and deer CWD prion isolates in the form of 10% brain homogenate in DBPS were incubated with CuCl2 or MnCl2 at 0.05, 0.5, and 5 mM (final concentration) for 1 week or 1 month at 22°C and 40°C. After incubation, prion samples were analysed for the amount of PrPRes using immunoassays and PMCA conversion capacity. To investigate CWD prion persistence in an environmentally relevant form, the CWD isolates were sorbed to soil clay minerals including kaolinite and montmorillonite followed with incubation or repeated dry-wet treatment in metal-supplemented conditions.

Results: The amount of unsorbed CWD PrPRes was significantly reduced when incubated with 0.5 or 5 mM CuCl2 for 1 month at 22°C or 40°C. In contrast, the amount of unsorbed CWD PrPRes remained unchanged mostly when incubated with MnCl2. However, the PMCA conversion capacity of CWD supplemented with CuCl2 was not significantly changed. The effect of copper and manganese supplementation on clay mineral-sorbed CWD is under investigation and will be available upon the completion of sample analysis.

Conclusions: In a soil free environment, CuCl2 had a greater impact on CWD than MnCl2 regarding the amount of PrPRes, indicating reduced resistance of CWD to the environmental degradation in a CuCl2-rich environment, though its conversion capacity might be maintained.

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14. Is weight gain an early sign of sporadic Creutzfeldt–Jakob disease?

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Aims: To investigate whether weight gain is part of the phenotypic spectrum of human prion disease.

Materials and Methods: We characterized a mouse-adapted human prion strain, MU-03, derived from a patient with T3MM (London classification) sporadic Creutzfeldt–Jakob disease (sCJD) (Ethics no. 0707227, 1111949 and 1413151). To validate the key observation of weight gain in this mouse model we examined the Australian National Creutzfeldt-Jakob Disease Registry (ANCJDR) patient database to determine if weight gain could be considered part of the phenotypic spectrum of human prion disease (Ethics no. 1341074 and 1647293). Both electronic database and hard copy patient files of 811 probable/definite cases of prion disease were investigated. These were compared to 256 cases referred to the ANCJDR as initially suspicious for prion disease but ultimately determined to have alternative diagnoses, the 'non-CJD' cohort.

Results: BALB/c male and female mice intracerebrally inoculated with the MU-03 strain unexpectedly displayed weight gain as the first disease sign. This disease

sign began to emerge halfway through the 80-week incubation period. Weight peaked at approximately 55 weeks post-inoculation which correlated with increased food intake. Examination of clinical records in the ANCJDR database led us to determine that there were significantly more CJD patients with a medical history of excess weight and also significantly more CJD patients with a profile of actively gaining weight and/or eating more during symptomatic disease when compared with non-CJD counterparts.

Conclusions: Unexpected weight gain and/or an increase in appetite could be an easily observable intrinsic but non-specific sign of prion disease in a subset of patients, which could aid in the early diagnosis of prion disease.

Funding: This work was financially supported by an Australian Government Research Training Programme Scholarship, a Melbourne Neuroscience Institute Strategic Research Training Program (RTP) Scholarship and philanthropic funding from the CJD Support Group Network (CJDSGN Memorial Grant in memory of: Frank Burton; CJDSGN Memorial Grant in memory of Catherine Heagerty; CJDSGN Memorial Ph.D. Scholarship in Memory of Frank Burton; PhD Top-up scholarship in memory of Primo Monaci; PhD Top-up scholarship in memory of Carol Willesee). The ANCJDR is funded by the Australian Federal Government Department of Health and Aged Care.

15 Cellular prion protein controls DNA damage response and fibrosis following kidney injury by interacting with epithelial growth factor receptor

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Aims: Cellular prion protein (PrPC) coded by the Prnp gene, is a kind of cell-surface copper-binding protein, anchoring on cell membrane lipid rafts through glycosyl phosphatidyl inositol anchor (GPI), and plays an important role in cell information transmission. PrPC is also expressed in the kidney. However, the role of PrPC in regulating fibrotic maladaptive repair of the injured kidney remains largely unknown. The aim of this study is to explore the role of PrPC in renal fibrosis follow injury.

Materials and Methods: Wild-type FVB mice, Prnp-/- mice were used for in vivo studies. Renal I/R injury model was induced by bilateral renal pedicle clamping for 35 minutes. Renal fibrosis models were induced by unilateral renal pedicle clamping for 30 minutes (UIR) and by unilateral ureteral obstruction (UUO). HK-2 cells were used for in vitro studies. HK-2 cells were treated with TGF-β induce EMT. In clinical study, 20 biopsy-proven minimal change disease (MCD) and 55 biopsy-proven tubular injury patients were included to explore the significance of PrPC in the diagnosis of renal injury.

Results: In this study, we observed increased expression of PrPC in renal proximal tubules in bilateral ischaemia-reperfusion (I/R), unilateral ischaemia-reperfusion (UIR) and unilateral ureteral obstruction (UUO) mice models. Deletion of PrPC exacerbated acute injury in renal tubules and subsequent renal fibrosis by persistently activating the epidermal growth factor receptor (EGFR) pathway. Notably, we identified increased PrPC/EGFR complex in endosomes of renal proximal tubular epithelial cells during UIR injury, and discovered a role for PrPC in regulating EGFR internalization. PrPC ablation also led to persistent activation of checkpoint kinase 1 (Chk1), resulting in maladaptive repair and subsequent suppression of cyclin-dependent kinase 1 (CDK1) in a time-dependent manner, inducing cell cycle arrest in the G2/M phase, in both UIR and UUO model. Furthermore, we observed secretion of PrPC into urine in injured tubules, and elevated urinary PrPC specifically in patients with renal tubular injury.

Conclusions: Our findings suggest that PrPC might regulate renal tubule repair by internalizing EGFR to stabilize the EGFR pathway, thereby alleviating G2/M cell cycle arrest in injured tubules. Moreover, urinary PrPC secreted by injured tubules might serve as a potential non-invasive biomarker for identifying renal tubular injury.

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16. The cellular prion protein antagonist PSCMA prolongs survival and reduces microglia dysregulation in the RML prion disease mouse model

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Aims: Targeting cellular prion protein (PrPC) is a promising therapeutic strategy for prion diseases, given that PrPC is the crucial factor in the misfolding cascade and the mediation of neurotoxicity. Despite the recent advancement of several drugs into clinical trial phases, continued screening and testing of pharmaceuticals remain necessary. Recently, PSCMA (poly [4-styrenesulfonic acid-co-maleic acid]) was shown to efficiently bind to the N-terminus of PrPC and block interaction with toxic Amyloid-β Moreover, PSCMA was shown to cross the blood-brain barrier through oral administration. The aim of this study was to evaluate its effect on PrPC and PrPSc levels and prion diseases progression.
Materials and Methods: The influence of PSCMA on PrPC levels was determined by western blot in Neuro 2A cells using different concentrations of PSCMA. The effects of PSCMA on cell viability and toxicity were evaluated using MTT and LDH assays. Based on this data, PSCMA dosing was selected for treatment of mice after infection with RML prions. PSCMA was administrated orally to RML mice starting either at 60 days post RML infection (60 dpi) or at 90 dpi, and continued until the endpoint. A subset of brains was collected at the preclinical stage, and others at the endpoint stage. To examine the impact of PSCMA in RML-infected mice, preclinical brains were analysed by RNA sequencing, western blot, histological and immunohistochemical (IHC) analyses, and immunofluorescence staining. Moreover, terminal tissues were assessed by HE and IHC.
Results: In vitro, PSCMA had a dose-dependent effect on PrPC and proteolytically shed PrP levels and exhibited no toxicity in N2a cells over three days at 1.5μM. In RML-infected mice, both PSCMA administrations at 60 and 90 dpi significantly extended the survival. PSCMA decreased PrPSc accumulation after treatment from 60 dpi at the preclinical phase. Additionally, the degree of spongiosis was reduced with both treatments at the preclinical phase. Interestingly, RNAseq data revealed only slight changes in overall gene expression upon treatment. While PSCMA treatment at both 60 and 90 dpi significantly reduced microglia dysregulation, no changes were noted in astrocyte numbers.
Conclusions: PSCMA prolonged the survival of prion-infected mice and improved microglia dysregulation even when the initial administration (90 dpi) was at the onset of symptoms. Thus, further studies should assess the therapeutic potential of PSCMA for treatment of human prion diseases.

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17 Transmission of vPSPr in macaque: an open gate to prion-like diseases ?

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Aims: A novel form of prion disease initially described in 2008 harbours very original features: reported in less than 50 cases worldwide to date, this prionopathy is characterized by the accumulation of abnormal prion protein with variable protease sensitivity depending on the genotype at PRNP codon 129, and subsequently called vPSPr. Their limited resistance, enforced by an incomplete transmissibility in rodent models, question the infectivity of these borderline prion strains. Moreover, their clinical expressions are often evocative of other neurodegenerative diseases (whose transmissibilities are also currently challenged) like Alzheimer's disease, fronto-temporal dementia or even amyotrophic lateral sclerosis, and concomitant deposition of other prion-like proteins is frequently reported. We aimed to assess the transmissibility of vPSPr cases in cynomolgus macaque, a model reputed as highly relevant of the human situation according to its close phylogeny.
Materials and Methods: Cynomolgus macaques were intracerebrally inoculated with brain samples issued from 1 M/M and 1 M/V vPSPr case respectively, and kept under surveillance throughout their incubation periods. At the onset of clinical signs, extensive histological, immunohistochemical and biochemical analyses were performed according to the techniques previously used on primates in our previous studies.
Results: Whereas the macaque exposed to M/M vPSPr inoculum remains healthy 14 years post-exposure, the macaque exposed to M/V vPSPr inoculum developed unusual neurological and behavioural disturbances after 8.5 years of silent incubation. The expected hallmarks of vPSPr were observed in this animal, including spongiform change and abnormal PrP depositions under different

forms. Unexpectedly, we also observed massive Aβ deposits in this mid-aged (13 years) macaque, a unique situation within our cohort of more than 50 over-ten years-old macaques, in which Aβ deposits may occur after 20 years of age but with different features, including co-staining with PrP, than in this animal.

Conclusions: The physiopathological mechanisms underlying the presence of Aβ deposits in both the brain of this animal recipient and its human donor remains to be elucidated. It may be either evocative of a concomitant presence of an age-related, but de facto transmissible, Aβ pathology with vPSPr in the donor patient, or a specific feature of vPSPr. At a time where the transmissibility of prion-like diseases is more than ever under question, this latter hypothesis would place these uncommon prionopathies at the intersection between prion and Alzheimer's disease, and maybe other neurodegenerative diseases as ALS and synucleinopathies according to the concomitant prion-like depositions described in some vPSPr patients.

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Grant number

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18. Zoonotic potential of moose-derived chronic wasting disease prions after adaptation in intermediate species

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Aims: Chronic wasting disease (CWD) is an emerging prion disease in Europe. To date, cases have been reported in three Nordic countries and in several species, including reindeer (Rangifer tarandus), moose (Alces alces) and red deer (Cervus elaphus). Cumulating data suggest that the prion strains responsible for the European cases are distinct from those circulating in North America. The biological properties of CWD prions are still poorly documented, in particular their spillover and zoonotic capacities. In this study, we aimed at characterizing the interspecies transmission potential of Norwegian moose CWD isolates.

Materials and Methods: For that purpose, we performed experimental transmissions in a panel of transgenic models expressing the PrPC sequence of various species.

Results: On first passage, one moose isolate propagated in the ovine PrPC-expressing model (Tg338). After adaptation in this host, moose CWD prions were able to transmit in mice expressing either bovine or human PrPC with high efficacy.

Conclusions: These results suggest that CWD prions can acquire enhanced zoonotic properties following adaptation in an intermediate species.

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19. Anti-chaperone activity of prion disease risk factor syntaxin-6

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Aims: Prions replicate via the autocatalytic conversion of cellular prion protein (PrPC) into fibrillar assemblies of misfolded PrP. While this process has been extensively studied in vivo and in vitro, non-physiological reaction conditions of fibril formation in vitro have precluded the identification and mechanistic analysis of cellular proteins, which may alter PrP self assembly and prion replication. We have developed a fibril formation assay for recombinant murine and human PrP (23–231) under near-native conditions (NAA) to study the effect of cellular proteins, which may be risk factors or potential therapeutic targets in prion disease with the aim to analyse their mechanism of action.

Materials and Methods: We assessed seeded and non-seeded aggregation PrP kinetics in NAA, sedimentation assays, and assessed aggregate morphology and co-aggregation by TAB/STORM super-resolution fluorescence microscopy. Interaction between syntaxin-6 and PrP was quantified by FRET imaging in scrapie-infected cell culture and the effect of syntaxin-6 on prion replication was quantified in PMCA assays using stx6 -/- brain homogenate.

Results: Previous genetic screening suggests that variants that increase syntaxin-6 expression in the brain

(gene: STX6) are risk factors for sporadic Creutzfeldt-Jakob disease (CJD). Analysis of the protein in NAA revealed counterintuitively that syntaxin-6 is a potent inhibitor of PrP fibril formation. It significantly delayed the lag phase of fibril formation at highly sub stoichiometric molar ratios in spontaneous aggregation assays, but did not alter seeded prion replication in PMCA.

However, when assessing toxicity of different aggregation time points to primary neurons, syntaxin-6 prolonged the presence of neurotoxic PrP species. Electron microscopy and super-resolution fluorescence microscopy revealed that, instead of highly ordered fibrils, in the presence of syntaxin-6 PrP formed less-ordered aggregates containing syntaxin-6. Both proteins interacted in scrapie-infected cells in perinuclear aggregates.

Conclusions: Our data strongly suggest that the protein can directly alter the initial phase of PrP self-assembly and, uniquely, can act as an 'anti-chaperone', which promotes toxic aggregation intermediates by inhibiting fibril formation.

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Reference Sangar et al. Elife. 2024, in press

20. Porins OmpC and OmpF of Salmonella enterica and Escherichia coli possess amyloid-forming properties

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Aims: Outer membrane proteins (Omps) of Gram-negative bacteria represent porins involved in a wide range of virulence- and pathogenesis-related cellular processes including transport, adhesion, penetration, and colonization of host tissues. Most Omps share a specific spatial structure called β-barrel that provides their structural integrity within the membrane lipid bilayer. Recent data suggest that Omps from several bacterial species are able to adopt an amyloid state alternative to their β-barrel structure. Amyloids are protein polymer fibrils with a specific spatial structure called cross-β that gives them an unusual resistance to different physicochemical influences. Different bacterial amyloids are known to be involved in host-pathogen and host-symbiont interactions and contribute to colonization of host tissues. Such an ability of Omps to adopt an amyloid state might represent an important mechanism of bacterial virulence.

Materials and Methods: The amyloid properties of the OmpC and OmpF porins from two species belonging to Enterobacteriaceae family, Escherichia coli and Salmonella enterica, were studied in vitro in detail using SDS-PAGE, transmission electron microscopy, x-ray diffraction, binding of amyloid-specific dyes and the method of circular dichroism (CD).

Results: We demonstrated that these porins form toxic fibrillar aggregates in vitro. These aggregates exhibit birefringence upon binding Congo Red dye, increase the fluorescence quantum yield of bound thioflavin T and show characteristic signals under x-ray diffraction. A significant change in the CD spectra of the samples during fibrillogenesis has been detected. For all these proteins, amyloid properties were also demonstrated across a whole range of parameters: detergent and protease resistance, thermal stability, interaction with specific amyloid dyes under polarization and confocal microscopy, characteristic images under transmission electron microscopy, as well as studies in the curli-dependent amyloid generator system (Belousov et al., IJMS, 2023).

Conclusions: Thus, we confirmed amyloid properties for OmpC of E. coli and demonstrated bona fide amyloid properties for three novel proteins: OmpC of S. enterica and OmpF of E. coli and S. enterica in vitro. The obtained data are important in the context of understanding the structural dualism of Omps, for studying the mechanisms of amyloidogenesis of Omps and their role in 'pathogen-host' interactions.

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21. Light Identification of Protein Suppressors (LIPS) as a New Technology to Screen for Genetic and Pharmacological Modulators of the Cellular Prion Protein

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Aims: Prion diseases are a class of neurodegenerative disorders that manifest in sporadic, inherited, or transmissible forms and are associated with the conformational conversion of the cellular prion protein (PrP), a cell surface glycoprotein primarily expressed in the central nervous system, into an aberrant form called PrPSc, which accumulates in the brains of affected individuals. PrPSc is a proteinaceous infectious particle (prion) capable of multiplying by directly recruiting PrP and causing its conformational rearrangement into new PrPSc molecules. It is increasingly evident that PrP plays a dual role in prion diseases, serving both as a substrate for PrPSc replication and a mediator of its toxicity. Data revealed that PrP may also act as a receptor for neurotoxicity transduction, particularly for oligomeric forms of amyloid β (Aβ) peptide and alpha-synuclein, critical players in Alzheimer's and Parkinson's diseases. These observations have significant therapeutic implications, suggesting that the genetic or pharmacological suppression of PrP expression may provide therapeutic benefits in various neurodegenerative conditions. Here, we aimed to deploy our cross-disciplinary expertise in computational, chemical, and biological sciences to develop an imaging-based method to rapidly quantify PrP expression, trafficking, and degradation in real time. We employed this assay to screen for genetic and pharmacological modulators of PrP.
Materials and Methods: We engineered a bimolecular fluorescence complementation assay named Light Identification of Protein Suppressors (LIPS), specifically designed to detect early events in PrP biogenesis, trafficking, and recycling in real time. The method was optimized to enable the ultrarapid screening of genetic or pharmacological libraries.
Results: We utilized the LIPS technology to screen a CRISPR library of approximately 1,000 RNA-binding proteins, a compound library of more than 2,000 FDA- and EMA-approved drugs, and an in-house collection of 160 natural extracts. We identified several genes, small molecules, and natural metabolites capable of suppressing PrP expression or promoting its delocalization to lysosomal degradation directly from the endoplasmic reticulum.
Conclusions: Our study highlights the LIPS assay's powerful ability to uncover genetic and pharmacological modulators of PrP, opening exciting possibilities for applying this innovative technology to other disease-related proteins. The computational integration of our genetic and pharmacological findings enables the construction of a CRISPR-drug perturbational map, defining novel pathways and therapeutic agents to safely and effectively modulate PrP expression. This effort lays the groundwork for developing therapeutic interventions for prion diseases and potentially other neurodegenerative disorders, offering promising avenues for future research and clinical applications.
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22.Vaccines that imitate the structural epitopes on α-synuclein fibrils offer protection against Parkinson's disease

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Aims: The age-related progressive aggregation of soluble α-synuclein into toxic oligomers and insoluble amyloid fibrils leads to neurodegenerative diseases such as Parkinson's disease, Lewy body dementia, and multiple system atrophy, all of which currently have no cure. Since α-synuclein is a self-antigen, pathogenic α-synuclein aggregates do not trigger a strong immune response. Recent advances in structural biology have revealed the structure of α-synuclein fibrils, enabling the design of engineered protein fibrils that mimic the conformational epitopes present on the surface of α-synuclein fibrils and that could serve as effective vaccines against synucleinopathies.

Materials and Methods: HET-s is a soluble fungal protein that can form amyloid fibrils. We vaccinated TgM83+/− mice, a model for Parkinson's disease-like synucleinopathies, using HET-s(218–298) fibrils and four modified derivatives, each displaying a specific conformational epitope found on the surface of α-synuclein fibrils.
Results: The fibrillar vaccine candidates significantly extended the survival of immunized TgM83+/− mice by up to 38% following intraperitoneal challenge and 42% following intragastric challenge with α-synuclein fibrils. Fully immunized mice developed antibodies that recognized α-synuclein fibrils and brain homogenates from patients with Parkinson's disease, dementia with Lewy bodies, and multiple system atrophy.
Conclusions: Vaccine candidates that imitate conformational epitopes on pathological α-synuclein fibrils can induce immunity and protection against Parkinson's disease and other synucleinopathies.
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23. Rationally designed, structure-based vaccine candidates targeting Chronic Wasting Disease

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Aims: Chronic wasting disease (CWD) is the most infectious prion disease affecting cervids such as deer, elk, moose, and caribou. There are currently no prophylactic vaccines or therapeutic treatments, leading to its spread in many parts of North America, South Korea, as well as Scandinavia. Based upon the theoretical four-rung β-solenoid (4 RβS) model of the infectious prion protein (PrPSc), our lab has developed a protocol using a modified fungal prion protein as an innocuous scaffold to create a rationally designed, structure-based vaccine candidate (YEG-Sc-1), which demonstrated efficacy when used to immunize a genetic prion mouse model. Our study now aims to quantify the immune response of YEG-Sc-1 in both Rocky Mountain elk and white-tailed deer, and then testing its efficacy in white-tailed deer.
Materials and Methods: Recombinant YEG-Sc-1 contains strategically placed amino acid side chains (alternating inwards versus outwards facing) to mimic predicted PrPSc surface residues. To quantify the immune response, Rocky Mountain elk (Wyoming) or white-tailed deer (Colorado) were immunized with YEG-Sc-1 and alum adjuvant in a double-blinded manner. Elk were given either a high (200 μg, n = 4) or low (100 μg, n = 4) dose, while deer were given 200 μg as the priming dose with 100 μg boosters, with (n = 3) or without (n = 4) a second vaccine targeting PrPC. Pre-immune sera were collected before inoculation and post-immune sera 3 or 4 weeks after final boost for elk and deer, respectively. An elk control group received no vaccine (PBS, n = 3), and a single deer acted as a control, also receiving no vaccine. YEG-Sc-1 efficacy testing in deer is currently on-going, using the same prime/boost schedule and antigen/adjuvant combination.
Results: Both elk and deer immunized with YEG-Sc-1 developed robust antibody titres, while control animals had no appreciable antibody titres as show by indirect enzyme-linked immunosorbent assays (ELISAs). The high dose (200 μg) elk group had slightly higher antibody titres overall, but were otherwise comparable to the low dose (100 μg) group. Deer that received either YEG-Sc-1 alone or both vaccines developed significantly higher antibody titres. The post-immune sera from YEG-Sc-1 immunized elk preferentially recognized brain tissue from transgenic elk CWD mice over its uninfected counterpart in 75% of the animals in competition ELISAs.
Conclusions: YEG-Sc-1 can elicit robust and PrPSc-specific antibody titres in both elk and deer. Efficacy trials in deer will aid in determining whether YEG-Sc -1 has potential as a bona-fide CWD vaccine.
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24. Curcuma phaeocaulis Valeton (Zingiberaceae) extract and compounds are efficacious to modulate prion propagation

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Aims: Evaluation of anti-prion efficacy of Curcuma phaeocaulis Valeton (Zingiberaceae) and identification of active compounds.
Methods and Results: Here, we report that Curcuma phaeocaulis Valeton (Zingiberaceae) (CpV) extract is effective in reducing prion aggregation and propagation in both in vitro and in vivo models. CpV extract inhibited self-aggregation of recombinant prion protein (PrP) in a test tube assay and decreased the accumulation of scrapie PrP (PrPSc) in ScN2a cells, a cultured neuroblastoma cell line with chronic prion infection, in a concentration-dependent manner. CpV extract inhibited prion infection in cultured cells as demonstrated by the modified standard scrapie cell assay. CpV extract also modified the course of the disease in mice inoculated with mouse-adapted scrapie prions, completely preventing the onset of prion disease in three of eight mice. PrPSc accumulation in the brain and spleen of mice was statistically significantly reduced. Furthermore, Biochemical and neuropathological analyses revealed a statistically significant reduction in PrPSc accumulation, spongiosis, astrogliosis, and microglia activation in the brains of mice that avoided disease onset. Next, We endeavoured to identify the chemical constituents of CpV extracts and discover potential anti-prion active compounds. Utilizing centrifugal partition chromatography (CPC), major constituents were isolated from the n-hexane (HX) fraction of the extract in a single step. Spectroscopic analysis confirmed the presence of curcumenone, curcumenol, and furanodienone. Subsequent efficacy testing in a cell culture model of prion disease identified curcumenol and furanodienone as active compounds. This study suggests the potential of natural products in finding effective treatments against prion diseases.
Conclusions: CpV extract has an anti-prion effect that inhibits the aggregation and propagation of prions. Two anti-prion active compounds (curcumenol and furanodienone) were identified in the CpV extract.
Funding: aMinistry of Education, bMinistry of Health & Welfare
Grant number: aNRF-2020R1A6A1A03042854, bHI16C1085
Acknowledgement: The authors would like to thank Taeyeon Kim for technical assistance in animal experiments.

25. Tear fluid RT-QuIC for prion disease diagnostic

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Aims: The main objective of our study is to modifying the RT-QuIC protocol for detecting PrPSc in less invasive biological fluids, tear fluid, for diagnosis of prion diseases. Based in these objectives, we formulated 2 tasks. The first task is to identify the most accurate rec PrP substrate for prion disease diagnosis. The second task is to modify the RT-QuIC assay protocol for the detection of PrPSc in tear fluid.
Materials and Methods: We modified our RT QuIC protocol by using a novel recombinant PrP substrate exhibiting a higher seeding efficiency through the introduction of the E200K PRNP mutation in the fulllength human PrP sequence and tested tear fluids from prion disease patients and controls.
Results: Using tear fluid, the assay was positive in 8 out of 9 sporadic CJD, 3 out of 4 genetic prion disease patients, and none of 26 control individuals. The results were validated in a consecutively acquired cohort, being positive in 5 out of 6 prion disease patients and negative in all 68 controls across the full spectrum of various neurological disorders. Of interest, the test was positive in tear fluids from 6 out of 8 asymptomatic PRNP mutation carriers.
Conclusions: Our study demonstrates that PrP seeding activity can be detected in prion disease patients using non-invasively obtained TFs. The test opens new avenues for early diagnosis and potentially for follow-up studies.
Funding: MS is supported by the CJD Foundation "Validation of a non-invasive diagnostic test for prion diseases using tear fluid". The study was funded by the Robert-Koch-Institute through funds from the Federal Ministry of Health.
Grant number: 1369–341
Acknowledgement

26. Experimental Challenge of Goats to determine genetic resistance to Classical Scrapie

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Aims: Classical Scrapie (CS) is a transmissible spongiform encephalopathy affecting sheep and goats. The susceptibility/resistance of sheep to CS is determined genetically by different PrP alleles. Transmission of CS to mice has revealed multiple strains. However, CS in goats is less understood in terms of genetic susceptibility to the disease, strain diversity and their relationship to PrP genotype.

Here we present data from CS transmissions to transgenic mice from goats that were either genetically resistant or susceptible to CS and were challenged with CS from a pool of natural cases from Cyprus [1]. The objective of these transmissions is to determine the CS strain isolated from the Cypriot goats.

Materials and Methods: A total of 21 (11 PO and 10 IC challenged) goats at clinical endpoint, or end of study for non-clinical cases (all of the PO inoculated goats, except N146N) were selected representing genetically susceptible (N146N) or resistant (N146S, N146D, D146D, S146S) animals. Brainstem homogenates were used to inoculate 8 transgenic mice (tg338, which overexpress an ovine VRQ PrP sequence, and tgShpXI, which overexpress an ovine ARQ PrP sequence), per inoculum and per line. The tg338 bioassays have been completed, and the tgShpXI bioassays are still ongoing. Incubation Period (IP) has been analysed, and Lesion Profile (LP) and Immunohistochemical patterns are being analysed and will be discussed with the poster.

Results: All the IC challenged goat inocula propagated into tg338 mice, regardless of goat genotype, while only inocula from N146N PO challenged goats managed to transmit to tg338 mice. Mean survival time (ST) was 642 days post inoculation (DPI) for the isolate that propagated in the mice from orally challenged goats and 180 DPI for the isolate that propagated in the mice from IC challenged goats.

Conclusions: The data obtained thus far shows that the same strain was isolated from all IC challenged goats, irrespective of their genetic resistance/susceptibility to the disease, suggesting that the genotype-associated phenotypic variability observed in the goats was probably due to the different PrP genetic background of the animals. The inoculation route of the goats led to two distinct disease phenotypes after passage in tg338 mice. This raises questions regarding bioassay experiments using a pool of infectious material, particularly of multi-strain TSE such as CS or CWD. Single strain TSE, such as atypical scrapie may be less affected.

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27. Autophagy regulates α-synuclein trans-synaptic propagation and induces dopaminergic neuron functional deficits in drosophila

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Aims: As the main pathological protein of Parkinson's disease (PD) and related disorders, α-synuclein (α-syn) was shown to spread between cells and from peripheral tissue to the brain in a 'prion' like manner. Researches have shown that α-syn possesses a special interneuron propagation path via the synapses. However, the exact mechanism and modulators of this process remain unclear. In this study, we aim to directly visualize the trans-synaptic spreading of α-syn in monosynaptic connections and reveal the key regulators and mechanisms involved in α-syn cell-to-cell propagation.

Materials and Methods: We employed the transgenic drosophila model to directly visualize the trans-synaptic spreading phenomenon of α-syn. Meanwhile, we knock down key proteins involved in autophagy and proteomic process and investigate their modulation effects on α-syn spreading. Using proteomic analysis combined with high-resolution confocal imaging, we further study the sequential effect of α-syn spreading on dopamine system and motor function of drosophila.

Results: We observed that α-syn propagate via the monosynaptic connection of the olfactory receptor neuron (ORN) and projection neuron (PN) of transgenic drosophila. Meanwhile, downregulation of autophagy

flux with Atg7 knockdown facilitated the protein transfer. In addition, increased spreading of α-syn from PN to the dopaminergic neuron decreased the level of dopamine and induced motor deficits in drosophila. In the end, using proteomic analysis, we revealed that increased accumulation of α-syn in dopaminergic neurons interfered with the synthesize of dopamine through downregulation of GCH-1 enzyme.

Conclusions: We revealed that α-syn can directly transfer via synapse and decrease in autophagy flux facilitate the process. The overwhelming spreading of α-syn induce downstream effect of reduced dopamine synthesize and motor deficits in drosophila.

Our study has provided a model to directly visualize the trans-synaptic propagating phenomenon of α-synuclein in vivo and suggested autophagy as a key regulator in this process. With the mechanistic study, we bridged up the pathogenic process of α-syn 'prion' like propagation and the symptomatic outcomes of motor deficits in PD models.

Funding: This work is supported by the National Natural Science Foundation of China (81430025, 82361138574, 82371273 and U1801681), the Key Field Research Development Program of Guangdong Province (2018B030337001), the Swedish Research Council (2019–01551) and Svenska Sällskapet för Medicinsk Forskning (SSMF, P18-0194).

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28. The purely thermodynamic anti-prionic mode of action for the treatment of all neurodegenerative diseases, including CJD.

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Aims: Neurodegenerative protein-misfolding diseases, like Alzheimer's (AD), Parkinson's disease (PD) and Creutzfeld Jakob disease (CJD), are driven by prion-like self-replicating and propagating protein assemblies of Aβ, α-synuclein, prion protein and many more. The conformation that these proteins have in their aggregated state is thermodynamically more stable than their physiological monomer conformation, which is often intrinsically disordered. Therefore, we have developed all-D-enantiomeric peptide ligands that bind the monomeric protein of interest with high affinity, thereby stabilizing the physiological intrinsically disordered monomer structure by the free binding energy. These ligands are eliminating already existing toxic aggregates by disassembling them into harmless monomers. This purely thermodynamic driven mode of action is truly 'anti-prionic', because it eliminates already existing oligomers and fibrils, thus disrupting prion-like replication and propagation of toxic protein aggregates. I will summarize the current progress in realizing the anti-prionic MoA for the target proteins α-synuclein, Aβ and prion protein.

Materials and Methods: Atomic force microscopy (AFM), dynamic light scattering (DLS), size exclusion chromatography (SEC), surface plasmon resonance spectroscopy (SPR), nuclear magnetic resonance spectroscopy (NMR), and a clinical study with 20 patients with MCI (mild cognitively impairment) to mild dementia due to AD in a single centre, randomized, placebo-controlled, double-blind study with RD2. Eligible patients were blindly randomly assigned (1:1) to receive 300 mg RD2 per day or placebo for 28 days. Follow-up assessment took place on day 56. The trial is registered in EudraCT 2020–003416-27.

Results: Briefly, the all-D-enantiomeric ligand for α-synuclein, SVD-1a, disassembles preformed α-synuclein fibrils (PFF) as shown by AFM and DLS. SPR and NMR demonstrated picomolar affinity of SVD-1a to α-synuclein monomers, while keeping α-synuclein monomers in their physiological IDP conformation.

The ligand for Aβ, RD2, demonstrated ex vivo target engagement and disassembled Aβ oligomers obtained from brain tissue of former AD patients (Kass et al., Cell Rep. Med. 3, 100630, 2022). A clinical phase 1b, double-bind, placebo-controlled study with 20 mild cognitively impaired (MCI) and mild AD patients treated once daily orally with RD2 for four weeks with an additional four weeks follow-up period yielded good safety and tolerability. As demonstrated before in transgenic and non-transgenic AD animal models, patients treated with RD2 improved their short-term memory abilities significantly, as shown with the Word List assay of the CERAD battery of neurocognitive testing. A placebo controlled double-blind proof-of-concept phase 2 study with 270 patients treated orally over 12 to 24 months with RD2 at two different doses or placebo is ongoing.

First results for realizing the anti-prionic mode of action for the prion protein will be shown.

I will also acknowledge the many contributors of these drug developments that are too many to be included here in the abstract.

Conclusions: The unique anti-prionic mode of action for the treatment of AD, PD, CJD and other protein misfolding diseases is promising.

Funding: Sprin-D and Michael J Fox Foundation

Grant number

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29. CJD Surveillance in New Zealand – the first twenty-five years

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Aim: To present an overview of the first 25 years of formal CJD surveillance in Aotearoa New Zealand (NZ).

Materials and Methods: Formal CJD surveillance in NZ started in 1997 and is aided by several factors. NZ is a small country with a predominantly public health system providing access to specialist services, with well-networked clinicians. There is a general recognition that assurance regarding transmissible diseases in humans and animals is important, with agricultural exports a key contributor to the NZ economy. 'Suspected CJD' must be notified by clinicians to the CJD surveillance registry and a public health doctor under government regulations. Accepted surveillance diagnostic criteria have been used. New Zealand CSF samples are analysed at the Florey institute in Melbourne, Australia, and pathological confirmation has been done at both the Australian and UK surveillance units.

Results: Over 25 years there were 57 definite, 71 probable, and 128 combined probable and definite cases of CJD. There have been no cases of variant CJD in NZ. There were two accidentally-transmitted cases and two genetic TSEs. The remaining 124 cases were classified as sporadic CJD. The NZ population grew from 3.781 to 5.245 million over this period and the incidence of CJD has remained within the expected rate. Surveillance sensitivity is very high when assessed against hospital discharge codes, national mortality data and requests for CSF testing.

Of the 128 definite and probable cases, 89 had a supportive MRI brain, 76 had a positive CSF 14-3-3 and 14 had a positive CSF RT-QuIC. Both CSF 14-3-3 and RT-QuIC were positive in 8. EEG was said to be supportive in 47.

Conclusions: CJD surveillance in NZ has been reassuring with no cases suspicious for variant CJD, and only two cases of accidently-transmitted CJD. This is despite earlier concern of a potentially higher rate of accidentally-transmitted CJD relating to pituitary hGH exposure. Genetic CJD has only rarely been identified in NZ with a historic lack of PRNP gene sequencing but there has been a recent improvement in pre-mortem DNA extraction from peripheral blood. Known inequities in NZ healthcare, particularly for Māori and Pacific peoples, need to be considered for the NZ context.

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30. A novel prion species identified in familial Creutzfeldt-Jakob disease linked to substitution of glutamic acid with lysine at residue 200 of prion protein

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Aims: To explore the molecular mechanism underlying a group of cases with genetic Creutzfeldt-Jakob disease (gCJD) linked to the most common PrP E200K mutation (gCJDE200K).

Materials and Methods: The brain tissues of 6 autopsy cases with atypical CJD phenotype were characterized with protein chemistry, neuropathology, in vitro seeding activity by real-time quaking-induced conversion and serial protein misfolding cyclic amplification assays as well as in vivo transmissibility by animal inoculation of prions from these autopsy cases.

Results: We report the identification of patients with atypical fCJDE200K, in which no PK-resistant PrPSc was detected by 3F4 with conventional and even PrPSc-enriched western blotting despite typical clinical phenotype and neuropathological changes. Instead, a peculiar PrPres was detected by an antibody against the C-terminal PrP220-231 called Anti-C. We also

detected such unique PrPres in the brain of typical fCJDE200K patients at lower levels, but not in sCJD cases, suggesting that this pathogenic PrP (PrPSc) molecule may represent the toxic PrPSc species specific to fCJDE200K. To determine whether it possesses seeding activity and infectivity associated with typical PrPSc, we further examined the brain samples with serial protein misfolding cyclic amplification (sPMCA), real-time quaking-induced conversion (RT-QuIC), and animal transmission assays. Compared to typical sCJD and typical fCJDE200K, the atypical fCJDE200K exhibits similar PrPSc-seeding activities but shows lower infectivity.

Conclusions: Our findings suggest that the anti-C binding PrPSc represents a novel pathogenic PrP conformer that could be a novel toxic PrPSc in fCJDE200K.

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31. Developing enhanced RT-QuIC for detection of serum tau-seeding activity as a diagnostic biomarker of Alzheimer's disease

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Aims: Although several biomarkers with high clinical value, such as brain imaging and body fluid analysis, have been developed, reliable detection of the pathological prion-like seeding activity (SA) of neurotoxic tau in body fluids and peripheral tissues as a diagnostic biomarker for Alzheimer's disease (AD) has remained elusive. The aim of our current study was to develop non-invasive and cost-effective methods for detecting misfolded tau, one of the two key pathological proteins crucial for early and differential diagnoses of AD.

Materials and Methods: Brain tissues from AD and non-AD cadavers confirmed neuropathologically were used to establish our tau-seed amplification assay utilizing ultrasensitive real-time quaking-induced conversion (RT-QuIC) technology. Serum and plasma samples were collected from patients clinically diagnosed with AD, mild cognitive impairment (MCI), and healthy controls (HC). Misfolded tau in the sera of subjects was enriched using immunoprecipitation (IP) with anti-tau antibodies prior to the RT-QuIC assay as seeds, allowing for the measurement of its prion-like SA. Recombinant tau fragments, specifically human tau fragments 3 R-cysteine-free (3 RCF) and 2RCF, purified from E. coli, were used as substrates in the assay. The detection efficacy of our RT-QuIC method was further evaluated against the single molecule array (Simoa) and brain imaging techniques. Simoa was employed to determine the levels of Aβ42/40, phosphorylated tau (p-tau)181, p-tau217, neurofilament light (NfL) chain, and glial fibrillary acidic protein (GFAP) in the serum, plasma and cerebrospinal fluid (CSF) samples from the same patients. Additionally, Aβ- and tau-PET results of some patients were collected to validate our RT-QuIC findings.

Results: Using brain homogenates AD and non-AD cadavers, along with preformed fibrils (PFF) of recombinant 3 RCF as seeds, we first established and optimized the RT-QuIC assay with a substrate mixture of 2RCF and 3 RCF to detect tau-SA. By titrating misfolded tau from AD brain homogenates or recombinant tau PFF spiked into normal human sera, our RT-QuIC assay successfully detected tau-SA at concentrations as low as 10–9 of diluted AD brain homogenate and 1 pg/mL of PFF. Western blot analysis of brain homogenates showed significantly higher levels of p-tau in AD compared to non-AD samples. The RT-QuIC assay of complexes enriched from sera using anti-tau antibodies showed increased tau-SA in AD patients compared to non-AD controls (76,708.9 ± 19,529.6 vs 46,898.6 ± 13,865.4, $p = 0.0027 < 0.005$). Additionally, the lag time of RT-QuIC reaction was shorter in AD compared to non-AD samples (41.7 ± 11.2 vs 59.0 ± 18.4, $p = 0.0282 < 0.05$). Simoa analysis revealed that levels of plasma and CSF p-Tau217 were significantly higher in AD patients than in non-AD controls (plasma: 1.379 ± 0.873 vs 0.239 ± 0.259, $p = 0.0004 < 0.0005$, AUC: 0.988; CSF: 61.63 ± 44.85 vs 21.38 ± 25.29, $p = 0.0051 < 0.01$, AUC: 0.900). Similarly, the amounts of p-Tau181 in plasma and CSF were significantly higher in AD patients than in non-AD controls (plasma: 44.1 ± 15.2 vs 5.9 ± 5.7, $p < 0.0001$, AUC: 0.996; CSF: 905.1 ± 507.3 vs 309.4 ± 150.9, $p = 0.005 < 0.01$, AUC: 0.958). The levels of GFAP were also dramatically higher, while the ratio of Aβ42/40 was lower in AD plasma samples compared to non-AD controls (GFAP: 198.6 ± 102.1 vs 59.7 ± 38.1, $p = 0.0007 < 0.001$, Aβ42/40: 0.066 ± 0.017 vs 0.084 ± 0.018, $p = 0.0264 < 0.05$). In contrast, there were no significant differences in

plasma levels of NfL, Aβ42, and Aβ40 between the AD and non-AD groups ($p > 0.05$). The collection and evaluation of brain imaging results for these patients are currently in progress.

Conclusions: Our study suggests that elevated tau-SA in serum may serve as a biomarker for distinguishing AD from non-AD controls.

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32 Towards real-time monitoring of prion infection: genetic code expansion to enable site-specific bioorthogonal labelling of functional and prion-convertible cellular prion protein in live cells

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Aims: How infectious prion fibrils (PrPSc) enter cells and replicate is poorly understood. They require access to PrP substrate in a certain cellular or extracellular niche to grow. They then must undergo fission in a certain niche to generate infectious seeds that can somehow be passed on and spread among cells. To gain further insights into these events, we aimed to capture them in real time using total internal reflection fluorescence (TIRF) microscopy. This required fluorescent labelling of the cellular prion protein (PrPC) in a way that can support prion propagation (conversion of PrPC into PrPSc) during live cell imaging, which current antibody- or fluorescent fusion protein-based methods cannot warrant.

Materials and Methods: We expanded the genetic code of prion-susceptible mouse neuroblastoma cells, by transfecting them with plasmids coding for orthogonal pyrrolysyl-tRNA synthetase (PylRS) and its cognate tRNA (PylT) from methanogenic archaeabacteria. The amber stop codon (UAG in RNA) in these archaea codes for a non-canonical amino acid (ncAA) pyrrolysine, so certain lysine-derivative ncAAs can be site-specifically incorporated into PrP by so-called amber suppression. We used ncAAs containing different dienophile groups for rapid click reactivity with various self-quenched tetrazine (diene)-bearing fluorophores, that switch from a dark to a bright state upon clicking.

Results: We generated PrPamb constructs with the amber codon inserted at different locations within the PrP gene and successfully delivered them on different plasmids to the neuroblastoma cells with expanded genetic code. We cultured the transfected cells in the presence of ncAAs and our PrPamb constructs were abundantly expressed, with normal PrPC distribution, as detected with confocal and TIRF microscopy after live click-tagging. This indicates high non-canonical amber codon readthrough rates with the translation terminating at the natural stop signal in our system. Prion propagation potential of our PrPambs is currently being tested in Scrapie Cell Assays using PrP knock-down cells. So far, at least one PrPamb is apparently prion-convertible in this system.

Conclusions: We laid the foundation for a novel cell-based platform for live monitoring of prion infection. Our engineered PrPambs, with only a single amino acid modification, show physiological distribution and can be fluorescently labelled under native conditions in live cells within seconds. Furthermore, we identified a PrPamb that successfully reports prion infection. This system will be instrumental in identifying key stages of prion propagation, allowing for close examination using super-resolution, single-molecule localization microscopy, and providing deeper insights into the nanoenvironments where prions establish infection.

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33. Genome-wide CRISPR activation screen identifies BMP signalling pathway as mediator of prion uptake by cells

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Aims: The key molecular players in prion diseases onset and propagation are yet undiscovered, and human genetics so far did not identify any disease-associated gene other than PRNP itself. To overcome

this issue, we decided to apply CRISPR-based genetic screens to the study of prion disease mechanisms.
Materials and Methods: The Aguzzi lab generated 42′146 lentiviral vectors for arrayed or pooled ablation (CRISPRko), activation (CRISPRa) and epigenetic silencing (CRISPRoff) of each protein-coding human gene, grouped along functional and topological networks. The vectors combine quadruple non-overlapping guide RNAs (qgRNA) with selection markers and a fluorochrome. gRNAs were designed to minimize off-target effects and to be tolerant to most human polymorphisms.

In my project, I leveraged this advanced CRISPR toolbox to investigate the initial step in prion disease progression: the uptake of prions by healthy cells, which triggers their spread throughout the brain. I developed a robust FACS-based assay to detect the internalization of PrP aggregates and used it to screen for modifiers of the uptake of infectious recombinant AlexaFluor-488-labelled ovine PrPSc, (produced by Prof. J. Castilla's group at CIC bioGUNE in Bizkaia, Spain). SH-SY5Y cells, knocked out for human PRNP, were infected with the pooled CRISPR activation library and incubated with fluorescent PrPSc for 24 hours before being sorted via FACS into prion-positive and prion-negative cells.
Results: Initially, I conducted a proof-of-concept screen focused on membrane proteins that might serve as receptors or inhibitors of prion entry. Encouraged by the results, I expanded the investigation to the entire genome. The genes identified in the initial screen were validated in the genome-wide screen, demonstrating the reproducibility and robustness of our assay.

Through gene ontology and pathway enrichment analyses, we identified several pathways and modifiers, some of which had never been associated with prions before. Notably, the Bone Morphogenetic Protein (BMP) signalling pathway emerged as the most enriched. This pathway, which has recently been linked to neurological disorders, is highly conserved across species and plays a critical role in various developmental processes. Dysregulation of the BMP pathway can lead to developmental abnormalities or diseases.

The BMP pathway operates by binding soluble BMP ligands to transmembrane heterotetrameric Ser/Thr kinase receptors, which then phosphorylate cytoplasmic transducers (R-SMADs). These transducers move to the nucleus to mediate transcriptional changes in target genes. Our primary hit list includes members from all three major components of the pathway – ligands, transmembrane receptors, and SMAD effectors – as well as SMAD-responsive transcription factors like RUNX2. Interestingly, in my screen the R-SMAD proteins identified (SMAD1, SMAD5) enhanced prion uptake upon activation, while the inhibitory SMAD6 had the opposite effect.
Conclusions: I am currently focused on unravelling the molecular events that connect BMP signalling with both the uptake and establishment of prion infection in prion-permissive cell lines.
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34. Detection limitations of prion seeding activities in blood samples from patients with sporadic prion disease

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Aims: Human prion diseases (HPDs) are fatal neurodegenerative disorders characterized by abnormal prion proteins (PrPSc). However, the detection of prion seeding activity in patients with high sensitivity remains challenging. Even though real-time quaking-induced conversion (RT-QuIC) assay is suitable for detecting prion seeding activity in a variety of specimens, it shows lower accuracy when whole blood, blood plasma, and blood-contaminated tissue samples are used. In this study, we developed a novel technology for the in vitro amplification of abnormal prion proteins in HPD to the end of enabling their detection with high sensitivity known as the enhanced quaking-induced conversion (eQuIC) assay.
Materials and Methods: Three antibodies were used to develop the novel eQUIC method. Thereafter, SD50 seed activity was analysed using brain tissue samples from patients with prion disease using the conventional RT-QUIC assay and the novel eQUIC assay. In addition, blood samples from six patients with solitary prion disease were analysed using the novel eQuIC assay.
Results: The eQuIC assay, involving the use of three types of human monoclonal antibodies, showed approximately 1000-fold higher sensitivity than the original RT-QuIC assay. However, when this assay was used to analyse blood samples from six patients

with sporadic human prion disease, no prion activity was detected.

Conclusions: The detection of prion seeding activity in blood samples from patients with sporadic prion disease remains challenging. Thus, the development of alternative methods other than RT-QuIC and eQuIC will be necessary for future research.

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35. Detection of Pathological Prion Protein in Milk Samples from Scrapie Naturally Infected Sheep by Real-Time Quaking-Induced Conversion assay

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Aims: One of the major challenges in managing TSEs in animals is the lack of validated and sensitive intravitam assays. The high sensitivity of Real-Time Quaking-Induced Conversion (RT-QuIC) in amplifying PrPSc across various biological matrices suggests that it is an ideal method for this purpose. However, it remains ineffective on blood and impractical for cerebrospinal fluid (CSF) due to collection challenges at the flock level. Given the established involvement of milk in prion transmission, the study aimed to develop RT-QuIC conditions for the detection of PrPSc in milk obtained from naturally Scrapie-infected sheep.

Materials and Methods: ~50 ml of milk samples were collected from lactating sheep belonging to an Italian outbreak of classical Scrapie. TSE diagnosis was performed on obex by rapid test (IDEXX HerdChek BSE-scrapie antigen test kit EIA rapid test) and western blot. Sheep brain tissues affected by Scrapie resuspended at 10% (weight/volume) in homogenization buffer were used for preliminary spiking experiments in milk collected from uninfected scrapie sheep and as positive controls in RT-QuIC reactions. Milk samples were added with an equal volume of isopropanol/butanol (1:1 v/v) and centrifuged at 13,000 g for 30 min. Supernatants were discarded and the pellets were suspended in 0.1% of SDS/PBS solution or in lysis buffer (N-lauroyl sarcosine) and assayed neat or diluted from 1:10 to 1:1000. RT-QuIC tests were performed according to the protocol previously reported by Favole et al (2019).

Results:The analysis demonstrated that two rPrP substrates (rHa 90–231 and BV 23–231) can sensitively detect Scrapie PrPSc spiked in diluted milk. Specifically, rHa PrP 90–231 exhibited rapid reactions with lag phases comparable to reactions seeded with Scrapie brain homogenates. Furthermore, the precipitation protocol using an isopropanol/butanol solution enabled the detection of seeding activity associated with the presence of PrPSc in RT-QuIC tests, with a latency phase of 20–30 hours when applied to 10 mL of individual milk samples collected from 2/2 Scrapie naturally infected sheep.

Conclusions: These data confirm the secretion of prions within milk during the early stages of disease progression and a role for milk in prion transmission. Furthermore, the application of RT-QuIC to milk samples offers a non-invasive methodology to detect scrapie during preclinical/subclinical disease.

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36. White Matter Integrity Involvement in Preclinical Stage of Familial Creutzfeldt-Jakob Disease: A Diffusion Tensor Imaging Study

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Aims: To explore the patterns of white matter (WM) alterations using diffusion tensor imaging (DTI) in the preclinical stage of familial Creutzfeldt-Jakob disease (fCJD) .

Materials and Methods: 7 asymptomatic carriers of PRNP G114V mutation and 6 non-carriers from the same fCJD kindred were recruited at baseline. Follow-up was obtained in 7 asymptomatic carriers and 2 non-carriers 2 years later. We also included 10 symptomatic CJD patients and 10 age-and gender-matched healthy controls out of the kindred to observe the overlapping patterns of WM between asymptomatic carriers and symptomatic CJD patients. All subjects received clinical, neuropsychological assessments, electroencephalogram (EEG) tests, and DTI at baseline and follow-up. Tract-based spatial statistics (TBSS) was used in DTI study for whole-brain voxel wise analysis of fractional anisotropy (FA) and mean diffusivity (MD) in WM. Results were compared in three groups: baseline carriers against non-carriers (baseline analysis), changes after 2 years in carriers (follow-up analysis), and differences between symptomatic CJD patients and healthy controls (CJD patients analysis).

Results: Neither the carriers nor the non-carriers develop any neurological symptoms during 2 years follow-up. Baseline analysis showed no group differences between carriers and non-carriers in MD and FA. Follow-up analysis showed significant increased MD in left inferior fronto-occipital fasciculus, left uncinate fasciculus, bilateral superior longitudinal fasciculus, and bilateral corticospinal tract ($p < 0.05$), among which increased MD in bilateral superior longitudinal fasciculus and right corticospinal tract overlaps the pattern of CJD patients.

Conclusions: Integrity involvement within multiple WM tracts could be detected in preclinical stage of fCJD.

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37. PRNP contributes to demyelination by affecting neuroinflammation through microglia-associated TREM2-DAP12 axis

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Aims: To study the role of PRNP on central nervous myelin and demyelination in cuprizone-treated animals.

Materials and Methods: Mice were fed with 0.4% cuprizone, a copper-chelator for six weeks, and weighed weekly. The motor function of mice was evaluated by open field test and rotarod test. The demyelination and vacuolation of myelin were evaluated by luxol fast blue stain with the brain tissue sections, and the changes of myelin basic protein (MBP) were detected by immunohistochemistry and western blotting. Microglia, astrocytes and oligodendrocytes were observed by immunofluorescence. Western blotting and RT-qPCR were used to detect immunoinflammatory response related factors. RNA-seq, RT-qPCR, western blotting and immunofluorescence were used to detect gene expression and changes in proteins associated with microglia.

Results: We made the following findings in the current study: (1) Weight loss was detected in all 3 types of mice exposed to cuprizone diet, among which the wild-type mice were most significant. (2) The decrease in the motor function was mainly observed in female wild-type mice but not in OPR-deletion and PrP-knockout female mice and all 3 lines of male mice. (3) Wild-type mice fed with cuprizone showed decreased expression of MBP along with myelin loss, but mice with OPR-deletion and PrP-knockout could be alleviated to a certain extent. (4) Wild-type mice fed with cuprizone had activated microglia and astrocytes, while the activation response of microglia in OPR-deletion and PrP-knockout mice was attenuated. (5) Wild-type mice fed with cuprizone had fewer mature oligodendrocytes compared to OPR-deletion and PrP-knockout mice. (6) Wild-type mice fed with cuprizone generated more inflammatory mediators compared to the OPR-deletion and PrP-knockout mice. (7) The expression of TREM2 and DAP12 genes related to microglia in wild-type mice fed with cuprizone was upregulated, while it was downregulated in OPR-deletion and PrP-knockout mice.

Conclusions: PRNP mediates cuprizone-induced central nervous demyelination by upregulating TREM2-DAP12 activation of neuroinflammation associated with microglial responses.

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38. Chronic Wasting Disease: improving diagnostic surveillance in Italy by the Real-Time Quaking-Induced Conversion (RT-QuIC) Assay.

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Aims: CWD surveillance and diagnosis have become an important issue in Europe, since the disease was detected, in 2016, in Norway, mainly because, like classical scrapie, it is a contagious disease. Although the diagnostic methods for the active surveillance in bovine and small ruminants are able to detect the European CWD strains, we performed a retrospective study on the italian deer samples coming from the voluntary national surveillance plan, to compare the results obtained from the rapid test in use and the RT-QuIC method, well-known as an innovative approach for its extremely high sensitivity.
Materials and Methods: One hundred brainstem and medial retropharyngeal lymph nodes from Italian deers, found dead, were selected from those belonging to the italian surveillance system. All these cases were analysed by the HerdChek BSE-Scrapie Antigen Test (Idexx) and resulted negative for both tissues.

These samples were then analysed by RT- QuIC using two different substrates of recombinant prion protein (rPrP) and following a protocol applied for other prion diseases.
Results: The analyses revealed no seeding activity in the brainstem and lymph nodes in any sample included in this study, except in one animal, at the level of the brainstem, with both rPrP substrates.

The brainstem from this case was then submitted to confirmatory TSE analyses by Western Blot and Immunohistochemistry, resulting negative for both methods.
Conclusions: The detection of seeding activity in one sample is remarkable, considering that CWD has not been detected in Italy, so far. RT-Quic is recognized, in human and animal prion diseases, to be more sensitive compared to classical diagnostic methods, allowing to detect the presence of PrPSc very early in different biological matrices and in pre-clinic step. On the other hand, the seeding activity detected by the RT-QuIC method is not always predictive of infectivity: bioassays will be performed to better understand the health status of this animal.
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39. Alpha-synuclein exhibits binding affinities for various Tau isoforms

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Aims: A key characteristic of several neurodegenerative diseases is the accumulation and aggregation of amyloidogenic proteins, such as amyloid-beta and Tau in AD and alpha-synuclein (aSyn) in synucleinopathies. Many neurodegenerative diseases exhibit mixed pathological features. For example, Lewy bodies have been observed in tauopathies, while neurofibrillary Tau tangles are found in synucleinopathies, indicating a possible coexistence or interaction between misfolded aSyn and Tau proteins. In this work, we focused on the role of Tau in synucleinopathies, supporting co-localization and interaction of aSyn and different Tau isoforms.
Materials and Methods: To investigate a direct interaction we subjected different Tau isoforms as well as recombinant human aSyn to surface plasmon resonance spectrometry (SPR). SH-SY5Y were treated with aSyn and different Tau isoforms under same conditions and immunofluorescence were conducted.
Results: We demonstrate the effect of Tau on the internalization of aSyn and the interaction between both proteins. SPR results presented aSyn as direct interaction partners to all six Tau isoforms. SH-SY5Y cells showed similar effects in co-localization.
Conclusions: Altogether, our experiments indicate a direct interaction and co-localization of aSyn with different Tau isoforms that could contribute to a better understanding of the pathological mechanism in synucleinopathies, which is important for future therapies or diagnostics.
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40. Arrayed CRISPR libraries for the genome-wide activation, deletion and silencing of human protein-coding genes

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Arrayed CRISPR libraries extend the scope of gene-perturbation screens to non-selectable cell phenotypes. However, library generation requires assembling thousands of vectors expressing single-guide-RNAs (sgRNAs). Here we show that, by leveraging massively parallel plasmid-cloning methodology, arrayed libraries can be constructed for the genome-wide ablation (19,936 plasmids) of human protein-coding genes, and for their activation and epigenetic silencing (22,442 plasmids), with each plasmid encoding an array of four non-overlapping sgRNAs designed to tolerate most human DNA polymorphisms. The quadruple-sgRNA libraries yielded high perturbation efficacies in deletion (75–99%) and silencing (76–92%) experiments and substantial fold changes in activation experiments. Moreover, an arrayed activation screen of 1,634 human transcription factors uncovered 11 novel regulators of the cellular prion protein PrPC, screening with a pooled version of the ablation library led to the identification of 5 novel modifiers of autophagy that otherwise went undetected, and 'post-pooling' individually produced lentiviruses eliminated template-switching artefacts and enhanced the performance of pooled screens for epigenetic silencing. Quadruple-sgRNA arrayed libraries are a powerful and versatile resource for targeted genome-wide perturbations.

41. Medical Research Council Prion Disease Rating Scale (MRC scale) scores of the Natural History Study by the Japanese Consortium of Prion disease (JACOP-NHS)

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Aims: To evaluate the activities of daily living (ADL) in patients at the time of enrolment in JACOP-NHS.
Materials and Methods: Age, sex, diagnosis, days from disease onset to enrolment, and total MRC scale score at enrolment were analysed using the dataset of JACOP-NHS from April 2017 to July 2024.
Results: Of 2077 cases enrolled in JACOP-NHS, there were 984 (418 male, 566 female cases) prion disease cases. The mean age of onset was 71.8 ± 10.8 years, the mean time from disease onset to enrolment was 153.1 ± 242.6 days, and the mean MRC scale score at enrolment was 8.3 ± 7.0. Diagnoses included sporadic Creutzfeldt–Jakob disease (CJD; 689 cases), V180I (187 cases), Gerstmann–Sträussler–Scheinker syndrome (GSS; 33 cases [29 P102L and four P105L]), M232R (32 cases), E200K (28 cases), fatal familial insomnia (FFI; four cases), V180I + M232R (two cases), and dura mater graft associated CJD, V210I, V203I, E200G, E196K, and 120-bp insertional mutation (one case each). Two cases were of undetermined classification. The mean time from disease onset to enrolment (days) and total MRC scale score (points) for each type of prion diseases were as follows: sporadic CJD 121.3 ± 150.8, 7.3 ± 7.1; V180I 165.5 ± 296.5, 10.6 ± 5.7; GSS 785.1 ± 524.2, 15.1 ± 6. 0; M232R 141.2 ± 163.4, 9.1 ± 6.9; E200K 138.3 ± 210.7, 6.9 ± 6.9; FFI 199.7 ± 139.1, 8.7 ± 3.7; and V180I + M232R 142.5 ± 15.5, 9.0 ± 6.0.
Conclusions: At the time of enrolment in JACOP-NHS, more than 4 months had elapsed since disease onset. Until the time of enrolment, the ADL (MRC scale scores) of patients already decreased to the medium to low level. For administration of investigational drugs in future, it is important to know MRC scale scores in much earlier stages from the onset of disease.
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42. Structure-Based Screening of Chemical Compounds Targeting α-Synuclein Fibrils for Diagnosis and Therapeutics of Synucleinopathies

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Aims: α-Synuclein (α-Syn) fibrillar aggregates are crucial pharmaceutical targets for diagnosis and therapeutics of synucleinopathies, emphasizing the need for compounds that can track them or inhibit their intercellular transmission. However, current assays, such as fluorescence saturation assays, only search for and validate compound binding to fibrils without any binding pattern information.
Materials and Methods: Here, we developed two structure-based high-throughput competition screening assays: the BF227 competition assay (BA) for tracers and the Heparin competition assay (HA) for inhibitors, based on insights into how chemical binders interact with α-Syn fibril pockets.
Results: These assays yielded promising hits, which were confirmed through cryogenic electron microscopy (cryo-EM) and surface plasmon resonance (SPR) by validating their binding to specific pockets. Furthermore, BA ligands (BA1-1 and BA1-2) effectively trace α-Syn fibrillar aggregates in neurons, mouse brain slices, and Parkinson's disease dementia (PDD) brain slices, while HA ligands (HA1-1 and HA3-2) inhibit the inter-neuronal spread of α-Syn fibrillar aggregates.
Conclusions: In conclusion, our structure-based assays enable rational screening to identify lead compounds, paving the way for their further optimization and potential clinical application as tracers and inhibitors.
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43. Developing vaccines for chronic wasting disease

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Aims: Chronic wasting disease (CWD) is an expanding prion disease in cervid species in North America that poses a serious threat to animal health. CWD is responsible for cervid population declines and has adverse economic effects on cervid farming, hunting and tourism industries. This asks for new tools to contain and manage CWD, and active vaccination is now widely considered a promising strategy. Our groups have pioneered prion vaccines and established a solid proof-of-concept in small and large animal models that vaccination is effective and provides protection in prion challenge models, including CWD. Here, we describe the development and characterization of nanovaccines and vector-based vaccines for oral and systemic prophylaxis of CWD, with an emphasis on reducing also prion shedding.
Materials and Methods: Vaccines target either disease-specific epitopes or the normal isoform of the prion protein. Purified recombinant deer proteins were encapsulated into poly-lactic coglycolytic acid (PLGA) nanoparticles. Commercial rabies vaccines served as a template for vector-based vaccine candidates. Mouse and cervid models are used for immunization, followed by CWD challenge. We study B-cell responses by ELISA and neutralization assays and prion disease pathogenesis by detection of PrPSc and incubation time. Amount of CWD prions in faeces is measured by prion amplification methods. We are testing vaccine candidates in transgenic and knock-in mice, as well as in deer, elk and reindeer.
Results: We developed and characterized nanovaccines and viral vector-based vaccines. For the nanovaccines, the

developed formulation demonstrated high encapsulation efficiency of both antigen and adjuvant and considerable stability, making it a promising platform for the oral delivery. Rabies is the gold standard for control of a wildlife infectious disease through vaccination. Two commercialized oral rabies vaccines are currently used in Canada and the United States (based on human adenovirus 5 and Vaccinia virus). Our current data show that our vaccine candidates are able to extend incubation time to prion disease and at the same time reduce prion shedding in knock-in mice infected with CWD prions when injected. When applied orally, nanovaccines provide both systemic and mucosal antigen-specific immunity. Ongoing studies in various mouse models of CWD infection and CWD challenge studies in WTD and elk will show how this affects prion disease and CWD prion shedding.

Conclusions: Our work shows that both nanovaccines and vector-based vaccines are good candidates for oral and injected vaccination, with the potential to delay prion disease and in parallel reduce prion shedding.

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44. 'Reactive microglia envelop viable neurons in prion diseases.

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The transformation of microglia and astrocytes into reactive states is recognized as an early event in the pathogenesis of prion diseases. Recent research indicates that both reactive microglia and astrocytes not only respond to prion infection but actively contribute to disease progression. This presentation will summarize our recent studies demonstrating that microglial activation is directly triggered by PrPSc, with the degree of activation being dictated by the sialylation status of the N-linked glycans on PrPSc. Since PrPSc sialylation is primarily determined by prion strain and, to a lesser extent, by brain region, the resulting neuroinflammatory responses follow strain- and region-specific patterns. We will present emerging data on the interplay between different brain cell types, highlighting the harmful effects of reactive astrocytes on neurons and endothelial cells of the blood-brain barrier. The presentation will also cover significant behavioural changes in microglia as the disease progresses, particularly focusing on alterations in their phagocytic activity, which play a key role in shaping disease outcomes. Our findings suggest that microglia undergo a shift in function from being primarily protective in the early stages to becoming detrimental in the later stages of the disease. We propose that at these advanced stages, neuroinflammation serves as one of the major drivers of prion disease progression.

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45. Decoding PrP misfolding: learning from nature to design the future

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Aims: Our study introduces a novel methodology for generating bona fide infectious prions de novo, allowing a comprehensive analysis of protein misfolding

across the largest collection of PrP sequences from mammalian species to date. We tested more than 400 different recombinant prion proteins, classifying them according to their spontaneous misfolding propensity and conformational variability.

Materials and Methods: We demonstrate the application of the Protein Misfolding Shaking Amplification (PMSA) technique, describing all necessary components for achieving efficient and reproducible genuine protein misfolding. We selected emblematic misfolded recombinant PMSA products to validate the authenticity of the infectious prions generated through our methodology.

Results: This method has successfully yielded infectious prions from an extraordinary range of species, including bat, deer, sheep, cow, mink, pig, human, dog, rabbit, and rodents, among hundreds of others. Our research encompasses the testing of more than 400 distinct recombinant proteins, ranking the misfolding propensities of all known wild-type prion proteins and analysing the theoretical stability of the globular isoforms in search of potential correlations with misfolding propensity. To validate the authenticity of the infectious prions produced through our method, we conducted comprehensive inoculation experiments that confirmed the infectivity of the newly generated prions from various species.

Conclusions: The notable efficiency of our method in replicating spontaneous prion misfolding provides a valuable opportunity to explore important aspects of prion diseases. Our study addresses fundamental questions in the prion research field, such as defining infectivity determinants, interspecies transmission barriers, and the structural influence of specific amino acids on misfolded proteins. The comprehensive framework we have established through this methodology and analysis will pave the way for significant progress in prion research and provide invaluable information for future diagnostic and therapeutic applications.

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46. Skin phosphorylated α-synuclein as a tissue biomarker for the diagnosis of α-synucleinopathies

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A reliable biomarker for α-synucleinopathies is urgently needed particularly at an early disease stage. In fact, the identification of these disorders is challenging as the classical symptoms, particularly in the early disease phase, can be indistinguishable from other neurodegenerative pathologies, such as tauopathies, of diseases of a different nature, for example vascular.

Due to the involvement of peripheral innervation, the possibility of identifying misfolded α-synuclein aggregates in the skin tissue, as phosphorylated α-synuclein (p-α-syn), has been shown to be possible using indirect immunofluorescence (iIF). Numerous articles from different centres and countries have demonstrated how this technique presents a high diagnostic accuracy in differentiating patients with α-synucleinopathies such as Parkinson's disease (PD) and dementia with Lewy bodies from healthy controls or different neurodegenerative diseases such as Alzheimer disease and tauopathies. In addition, some recent data show that iIF may be able to differentiate PD from multiple system atrophy (MSA) by searching p-α-syn in skin Schwann cells, which can be considered as a promising specific biomarker for MSA. Other important data achieved by this technique are those obtained in the prodromal stages of synucleinopathies. Numerous iIF studies demonstrated that skin p-α-syn can be detected in the in the prodromal stages, e.g., in idiopathic rem behaviour disease (RBD), which can precede the onset of central nervous system involvement by several years.

In this presentation, the main acquisitions of the iIF technique as a biomarker of α-synucleinopathies will be presented together with some technical aspects that still need to be implemented and improved to help the clinical application and favour a large-scale automation of this technique.

47. CJD Foundation

Maria Thacker-Goethe

Grants & Fellowships: Empowering Research. Inspiring Hope.

This session will explore patient's family passion for supporting research and how the research grant program enables recipients to share their work at family conferences, connect with families one-on-one, and be inspired in their work based on contact with families. the opportunity and impact of two funding programmes sponsored by the US-based CJD Foundation. The CJD Foundation Grant Program allows families to directly support prion disease research, including studies on diagnostics, disease mechanisms, and treatments. Each year the CJD Foundation issues a Call for

Research Grant Applications and asks the Scientific Advisory Committee to review and rate those studies. The CJD Foundation then awards research grants to the top-rated applications from around the US and the world. To date the CJD Foundation has funded nearly $5 million in prion disease research. Additionally, in January 2025 the CJD Foundation may award a fellowship award for a researcher who is in the early years of their career and is working under the direct supervision of a senior investigator, healthcare, or public health professional. This award will fund a training fellowship in an established laboratory/clinical/research setting that will provide a foundation for the applicant's independent research career.

48. Prion Neurotoxic Pathways

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Aims: To define the cellular and molecular mechanisms underlying prion neurotoxicity, and develop therapies to block these mechanisms.
Materials and Methods: To dissect the earliest synaptotoxic changes induced by prions, we have used mouse hippocampal neurons cultured at low density in the presence of a glial feeder layer. Results were confirmed by analysis of brain samples from prion-infected mice.
Results: We previously observed that purified PrPSc causes a rapid (with 4 hrs) retraction of postsynaptic dendritic spines with concomitant abnormalities in synaptic function, long before any compromise of neuronal viability. These effects were strictly dependent on neuronal PrPC expression. Within minutes, PrPSc caused by NMDA receptor-dependent Ca2+ influx and hypersensitivity to externally applied NMDA. This was followed by p38 MAPK activation and collapse of the actin cytoskeleton within dendritic spines. Using mouse PrPC mutants (G126V and V208M) that are conversion-resistant, as well as mouse neurons expressing hamster PrPC, and recombinant PrPSc forms with varying levels of infectivity, we have now shown that cell-surface PrPSc is directly responsible for initiation of the prion synaptotoxic signal. Consistent with this conclusion, compounds that prevent accumulation of PrPSc also block synaptotoxicity, but only if applied within the first few hours after exposure to PrPSc.

We have also carried out transcriptomic and phosphoproteomic analyses of PrPSc-treated hippocampal neurons, and processed the results using a chemoproteomics pipeline. This resulted in identification of three key protein kinases (CaMKII, PKC, and GSK3β), pharmacological inhibition of which blocked downstream synaptotoxic events. We hypothesize that newly generated, cell-surface PrPSc directly activates CaMKII and PKC via NMDA other cell surface receptors, resulting in translocation of these kinases to the postsynaptic density.
Conclusions: We have defined several of the earliest detectable molecular events leading to synaptic degeneration in neurons exposed to prions. Some of these events may occur prior to, and independently of chronic infection of the neurons.
Funding: U.S. National Institutes of Health
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49.α-synuclein Aggregation and Propagation in Parkinson's Disease

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Parkinson's disease (PD) is characterized by the degeneration of dopaminergic neurons accompanied by Lewy bodies, which are pathological aggregations of proteins and lipids. α-Synuclein (AS), a main component of Lewy bodies and glial cytoplasmic inclusions, plays a critical role in this process. Pathogenic mutations and multiplications of AS are known to cause familial PD, establishing AS as a key protein in synucleinopathies. AS is an amphipathic protein that weakly binds to lipid membranes and functions intracellularly. In familial PD, pathogenic mutations and overexpression of AS alter its membrane binding, leading to increased aggregation. AS also localizes to lipid rafts, membrane functional domains rich in sphingolipids and glycosphingolipids. Alterations in glycolipids are crucial for AS aggregation. For example, ceramide, produced from glucosylceramide by glucocerebrosidase (an

enzyme associated with Gaucher's disease), is implicated in PD development when this enzyme malfunctions. Similarly, impairments in phospholipase A2 group 6 (PLA2G6), which cleaves acyl groups from phospholipids, lead to PARK14, with autopsies of PARK14 patients revealing Lewy bodies. Therefore, changes in lipid metabolism and membrane lipid composition significantly contribute to AS aggregation.

Furthermore, AS oligomers are central to PD pathogenesis, disrupting membrane binding abilities and inducing aggregation in membranous organelles such as mitochondria, lysosomes, synaptic vesicles, and autophagosomes. These AS oligomers act as seeds for further AS aggregation, and detecting them may provide insights into diagnosing PD and understanding synucleinopathy mechanisms.

Using synuclein amplification assays, such as real-time quaking-induced conversion combined with immunoprecipitation (IP/RT-QuIC) and Protein Misfolding Cyclic Amplification (PMCA) assays, has detected body fluid and specimens of AS oligomers. These techniques leverage seeding properties to amplify small quantities of seeds. We employed RT-QuiC with immunoprecipitation assay (IP/RT-QuCI) and detected serum AS seeds in synucleinopathy, highlighting its potential as a diagnostic biomarker. Additionally, the aggregation, propagation propensity, and microstructures of fibrils obtained from IP/RT-QuIC revealed differences among various forms of synucleinopathy.

Our research focuses on lipid metabolism and AS aggregation to unravel the pathogenesis of PD. In this symposium, we will review the role of AS as a diagnostic biomarker and introduce our study on serum AS oligomer detection using IP/RT-QuIC assays. Furthermore, we will discuss the mechanisms of PD development, emphasizing the roles of lipid metabolism. Our aim is to provide a comprehensive understanding of PD pathogenesis and explore potential diagnostic avenues.

Funding

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50. Identification of critical gene targets in prion infection

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Aims: In prion diseases, misfolding of the prion protein (PrP), an abundantly expressed cell surface protein, results in the formation of infectious PrP conformers, known as prions. The link between the self-assembly of misfolded PrP conformers into higher-order complexes and prion pathogenesis is not fully understood. Our goal is to identify biological pathways with a role in prion propagation and their corresponding gene targets using a gene loss-of-function approach, followed by pathway analysis.

Materials and Methods: Our approach leverages highly efficient pools of small interfering RNAs (siPools) in combination with a modified Scrapie cell assay, designed to measure changes in cellular infection rates at high throughput. Validation of sixty siPools against distinct gene targets achieved mean knockdown efficiencies of 89 ± 6%, demonstrating the robustness and effectiveness of this method. We here report our detailed analysis of endocytosis pathways and protein interaction networks.

Results: We first examined how prion infection is affected by perturbing key modulators of endocytosis. To date, more than a dozen endocytosis pathways have been characterized, each with distinct yet overlapping mediators, including clathrin (CLTA), dynamins (DNM), Ras homolog family member A (RHOA), endophilins, cell division cycle 42 (CDC42) and ADP ribosylation factors (ARF). We assessed prion infection in a susceptible neuroblastoma cell clone (Gly70-myc PrP PK1-10) following the single knockdown of more than a dozen gene targets. Prion infection was blocked most effectively by transcriptional silencing of Cdc42 (67%), Dnm1/Dnm2 (54%) and Sh3gl1 (endophilin A2, 47%), while other mediators including RhoA (12%), Clta (0%), Arf6 (21%) and Rac1 (3%) had little or no effect. CDC42 mediates endocytosis of glycosylphosphatidylinositol-anchored proteins and fluid-phase uptake in cells. To further scrutinse the role of CDC42 in prion infection we examined gene candidates associated with the CDC42 functional protein interaction network (String). Notably, two genes involved in regulating actin polymerization – WASP-like actin nucleation-promoting factor (Wasl) and brain-specific angiogenesis inhibitor 1-associated protein 2 (Baiap2) – significantly blocked prion infection, with effect levels of 51% and 32%, respectively.

Conclusions: Prion infection is effectively blocked by transcriptional silencing of Cdc42, the main mediator of a clathrin-independent endocytosis pathway termed CLIC-GEEC. A potential contribution of WASL and BAIAP2 to the CLIC-GEEC endocytosis pathway is currently under investigation.

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Acknowledgement: We thank Christian Schmidt, Parvin Ahmed and George Thirlway for technical assistance with Automated Scrapie Cell Assays.

51. Distinct tau fibril types and their role in prion diseases

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Aims: Neurodegenerative disorders are often characterized by the co-deposition of different amyloidogenic proteins, usually associated with distinct proteinopathies. In prion diseases, tau amyloid deposits have been reported together with the classical deposition of PrPSc. However, the effects of this co-deposition on prion disease progression are still unclear. Previously, we have shown that treating chronically prion-infected cells with K18 tau fibrils reduce PrPSc levels. Here, we further investigate this phenomenon using a distinct type of tau fibrils that, compared to the K18 ones, include the sequence that forms the core of tau Alzheimer's disease filaments in vivo.
Materials and Methods: RML prion infected ScN2a cells were exposed to different fibril types obtained in vitro from tau recombinant proteins and cell lysates subjected to Proteinase K digestion to evaluate differences in prion level. Immunofluorescence was used to determine the cellular localization of tau fibrils after their internalization. The influence of tau fibrils on prion replication was assessed by RT-QuIC and ELISA.
Results: First, we show that the two tau constructs form fibrils with distinct biochemical and biological characteristics. However, these differences do not affect their ability to reduce the level of PrPSc in chronically infected ScN2a cells. Although they are actively internalized by cells through an energy-dependent pathway, tau fibrils are not dependent on this mechanism to reduce PrPSc levels. Their effect on prion load was linked to their ability to inhibit PrPC to PrPSc pathological conversion, not only in chronic phases, but also in early stages of prion infection.
Conclusions: Our data support the concept that the binding of tau fibrils to the PrPC located on the plasma membrane could stabilize the protein, hindering its conversion to PrPSc, and in turn leading to prion reduction loads. Overall, our results suggest that PrPC is a receptor not only for distinct amyloids, but also for different amyloid conformations of the same protein and indicate a role for tau fibrils in preventing PrPC to PrPSc conversion.
Acknowledgement: The authors wish to thank Micaela Grandolfo for technical support.
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52. Exploring the Mechanism of Rapid Neurotoxicity in Prion Disease

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Similar to other late-onset neurodegenerative disorders, prion diseases share common characteristics such as late-age onset, protein aggregation, and neurodegeneration. However, prion diseases are distinct from other neurodegenerative diseases because they can be transmitted and have a rapid disease progression. Despite extensive research spanning several decades, the exact neurotoxic mechanism of prion disease remains unknown. This lack of understanding hampers the development of effective therapeutic treatments. Recently, we established a neurotoxic model in mice based on the N-terminal region of the prion protein. Using this model, we conducted a wide range of analyses. In this talk, I will present the findings from these studies and discuss their relevance to prion disease, which may shed light on the underlying mechanism responsible for the rapid disease progression observed in prion disease.
Funding: This work was supported by internal funding from the Chinese Institute for Brain Research, Beijing (CIBR).
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53. The impact of microglia-astrocyte cross-talk on CNS prion disease pathogenesis

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Microglia, like tissue macrophages, are mononuclear phagocytes that remove dying/apoptotic cells, defend against pathogens and also provide homoeostatic support. Consistent with this, studies show that partial microglia deficiency/ablation accelerates prion disease and increases prion accumulation in the brain, suggesting that microglia provide neuroprotection by engulfing prions. Microglia proliferation and survival are dependent upon colony stimulating factor 1 receptor (CSF1R) signalling. We created Csf1rΔFIRE mice lacking the fms-intronic regulatory element within the Csf1r gene. These mice completely lack microglia, but retain brain-associated macrophages and most peripheral macrophage subtypes, and their brains develop normally without the deficits reported in other microglia-deficient models. We used these mice as a refined model to study the impact of complete microglia-deficiency on CNS prion disease. Complete microglia deficiency in Csf1rΔFIRE mice did not enhance the accumulation of prions in the brain, contrasting previous studies, indicating that microglial degradation of prions is redundant. The accelerated CNS prion disease in Csf1rΔFIRE mice instead coincided with earlier reactive astrocyte dysregulation. Our data suggest that microglia instead provide neuroprotection by moderating the deleterious effects of neurotoxic/neuroneglective reactive astrocytes, but lose this ability as they adopt a disease-associated microglia phenotype as the disease progresses. Since neurotoxic/neuroneglective reactive astrocytes contribute to the neuropathology in many CNS conditions and ageing, understanding how microglia limit this activity will have widespread application. This could identify novel treatments to limit the progression of prion diseases and other neurodegenerative disorders, and also help to counteract the adverse effects of ageing on the brain.

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54. Genomic analysis of human prion diseases

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Aims: Human prion diseases have strong genetic determinants of both risk and clinical phenotypes. In this presentation I will summarize work in recent years from a consortium of researchers who have collected samples from patients with probable or definite Sporadic CJD (sCJD) and Inherited Prion Diseases (IPD), predominantly in populations with European ancestries.

Materials and Methods: Genome wide association study (GWAS) in sCJD and IPD, multiple transcriptome and proteome-wide association studies and Bayesian genetic colocalization analyses (coloc) between sCJD risk association signals and multiple brain molecular quantitative trait loci signals. Systematic gene prioritization and nomination of prioritized sCJD risk genes with risk-associated molecular mechanisms. Some hypothesis testing in model animals.

Results: The main determinant of risk and clinical phenotype is the well known coding sequence of PRNP, however other risk loci and mechanism are becoming clear: increased expression of Syntaxin-6 and protein disulphide isomerase A4 in the brain, reduced expression of Mesencephalic Astrocyte Derived Neurotrophic Factor, and coding variation of Galactose-3-O-Sulfotransferase 1. These genes link together intracellular trafficking, sulfatide metablism and the unfolded protein response as relevant to human prion diseases. Results will be presented that include new sCJD GWAS samples, and an IPD GWAS.

Conclusions: Human genetic studies provides new insight into the molecular pathogenesis of human prion diseases. Whether these findings have any relevance to therapeutics requires the development of hypotheses about how these gene loci confer altered risk and testing in model systems.

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55. Experimental Transmission of Protease Sensitive Prionopathy (VPSPr) to Nonhuman Primates

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Aim: Transmission is one of the main characteristics of prion diseases. However, VPSPr, which is now presumed to be the sporadic form of the genetic CJD V180I, is incompletely, if any, transmissible in humanized mice, and transmission in bank voles 'does not replicate the complex VPSPr-PrPd profile' (Nonno). We strenghtened the panel of experimental transmission of this uncommon prion strain with cynomolgus macaque, considered a relevant model of human prion diseases.

Material and methods: Transmission was performed in two cynomolgus macaques (PrP MM genotype at codon 129). The animal exposed to the brain of a 75 years-old MV patient developed clinical symptoms after 8.5 years of silent incubation. Euthanasia was performed 4 months later for humane reasons. The other animal is still asymptomatic 14 years after exposure to the brain of a MM patient (68 years).

Subsequently, secondary transmission to transgenic mice overexpressing macaque PrP were conducted.

Histological and biochemical studies were performed as previously published.

Results: The known clinical data of the MV patient were limited to executive dysfunction and unsteady gait.

The MV VPSPr-exposed macaque presented several episodes of self-aggression, first focused on his left leg, which extended to the whole hindquarters. Abnormalities of both hind limbs sensitive conduction were recorded.

Among our different analyses, neuropathological examination showed a spongiform change diffuse in the grey matter with a special profile, associated to a reactive astrocytosis and massive neuronal vacuolation, particularly in the rarefied Purkinje cells. Abnormal PrPd consisted of a diffuse background of thin synaptic deposits mixed with mini aggregates (dots) and some small cortical fussy fibrillar plaques, not preserved after proteolysis, and never observed in primates exposed to other prion strains. At the opposite there was no granules or micro-plaques in the cerebellum although they were noted in the donor patient.

Three other facts must be emphasized: i/ the presence of massive amyloid deposits in the neocortex but not in the hippocampus, except the subiculum more or less associated to PrPd. ii/ the negativity of the staining with AT8, AT100, cytoplasmic TDP43. iii/ the inflammatory reaction observed in the lumbar dorsal root ganglia which may be correlated with the peripheral itching of the legs.

Conclusion: Conversely to classical prion transmission in primate, neuropathology exemplified here a tiny specific pattern of PrPd deposits reminiscent of VPSPr in human. Our observations in primate and secondary in mice sustain the transmissibility of (at least MV) vPSPr-related prion strain which extends the field of classical prion diseases.

Funding: This study has been performed on internal funding of the laboratory.

Grant number

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56. Sensitive detection of proteopathic seeds from surfaces, tissue biopsies and biofluids

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Aims: To report on our progress with sensitive detection of misfolded prion, α-synuclein and Tau on a variety of surfaces and in tissue biopsies and biofluids from neuropathologically confirmed as well as clinically assessed individuals with neurodegenerative diseases.
Materials and Methods: We tested biospecimens such as skin, intestinal biopsies, blood, urine, olfactory mucosa and cerebrospinal fluid as well as autopsy collected gastrointestinal tract and skin samples from prion diseases, synucleinopathy and tauopathy patients. We applied the prion, α-synuclein or 3 R/4 R K12 Tau real-time quaking-induced conversion (RT-QuIC) seeding amplification assays, sometimes in combination with a capture step, to estimate levels of proteopathic seeds in non-central nervous system peripheral specimens of diagnostic relevance. We also used the RT-QuIC reaction mix to detect these seeds on a variety of solid surfaces such as surgical tools.
Results: Our findings indicate that misfolded prion, α-synuclein and 3 R/4 R Tau are detectable by RT-QuIC in a variety of biospecimens, often during early disease stages. While biofluids such as blood and urine continue to be a challenge, detection in skin and intestinal biopsies as well as olfactory mucosa specimens was possible for most pathological seeds. While we found that ease of detection in tear fluids was influenced by the collection method used, the same was not true, for example, for olfactory mucosa samples collected using a variety of swabs. We also developed a user friendly and practical strategy to collect misfolded proteins from surfaces for RT-QuIC analysis.
Conclusions: Our work so far highlights peripheral accumulation of misfolded proteins outside of the brain with ample evidence of co-pathology and the usefulness and versatility of the RT-QuIC assays in pursuing ante-mortem diagnosis, post-mortem investigations and surface detection of neurodegenerative diseases related pathological proteins.
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57. The molecular interaction between α-synuclein and the prion protein and associated biological effects

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Aims: The interaction between α-synuclein (aSyn) and prion protein (PrP) has been implicated in the pathogenesis of synucleinopathies. However, the nature of this interaction remains poorly understood. Here, we aimed at detailing the molecular mechanisms involved in the interaction between the two proteins and at assessing the biological effects of the interaction.
Materials and Methods: We employed a combination of biophysical (NMR, SPR) and cellular assays to characterize the interaction between aSyn and PrP.
Results: Our results demonstrate that the C-terminal region of aSyn interacts with PrP in an orientation-specific manner through electrostatic interactions, and suggest that tryptophan residues on PrP may also bind to the tyrosine residues of aSyn through π–π interactions. We further demonstrate that PrP potentiates seeded aSyn aggregation kinetics, as evidenced by an increase in monomer incorporation rate in the real-time quaking-induced conversion assay. Additionally, we observed colocalization between PrP and phosphorylated aSyn, suggesting a possible role of PrP on aSyn phosphorylation. We also found that aSyn oligomers impair neuronal function by interacting with surface PrP.
Conclusions: Our findings provide valuable insights into the nature of the aSyn-PrP interaction and highlight potential therapeutic targets for synucleinopathies. In particular, targeting the C-terminal of aSyn or the N-terminal of PrP may constitute a promising strategy for preventing or reversing the effects of aSyn aggregation and spreading.
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58. 'Procrustean bed: forcing PrPC into the PrPSc shape. A perspective'

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Elucidation of the atomic structure of PrPSc has finally opened the possibility of understanding the mechanism of prion propagation. Contemplation of PrPSc

immediately suggests that it can template conversion of the disordered ~90-120 segment of an incoming PrPC molecule. The resulting decrease in entropy must be, without doubt, offset by the decrease in enthalpy derived from the extensive array of hydrogen bonds formed between this segment and the β strand-rich templating surface of PrPSc. Once this first templating step is completed, the folded domain (FD) of PrPC is left tethered on the conversion surface, onto which it needs to accommodate. However, the PIRIBS ~120-230 PrPSc surface is a rather inert 'procrustean bed', with few options to interact with the globular FD hovering above it. Therefore, it seems that the FD must lead the remaining steps of conversion, with a substantial role in changing its conformation to adapt it to the template. Specifically, the FD has to partially unfold before it can refold into the conformation of the template.

I will recapitulate published experimental evidence from others and unpublished data from my group supporting the notion that the tethered FD 'opens' through separation of its (β1-α1-β2) and (α2-α3) subdomains. The flatter subdomains can now collapse onto the templating surface, allowing formation of temporary or stable bonds with it. However, templating requires additional unfolding of specific regions within the subdomains. I will discuss data from thermally induced unfolding of recombinant bank vole PrPC (BvPrPC) tracked with solution NMR that aim at pinpointing the unfolding timeline. BvPrPC is a good choice for such studies. Bv PrPC is known to adapt to all known PrPSc strains and while it is conceivable that its sequence allows BvPrP fit in every known PrPSc conformation without steric clashes, a more parsimonious explanation is that BvPrPC is particularly adept to unfolding. Perhaps BvPrPC can explore a wider range of alternative unfolding routes as compared to other PrP sequences, thus avoiding steric traps along the conversion pathway.

All in all, I will contend that the available data suggest that PrPC, far from being a passive object of conversion, is an active subject in the process. Studying thermally induced PrPC unfolding should provide key information on the conversion process, but such studies need to take into account the existence of a PrPSc template avid to 'trap' unfolding intermediates, or otherwise they will inform on non-PrPSc PrP aggregate formation.

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59. The role of plasmin in PrPSc propagation

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Aims: The underlying mechanism for the differential metabolic processing observed for the prion protein (PrP) in healthy and prion-infected conditions has been a research topic for years. Foremost, the physiological α-cleavage of PrP interrupts a region critical for both toxicity and conversion of cellular PrP (PrPC) into its misfolded pathogenic isoform (PrPSc) by generating a glycosylphosphatidylinositol (GPI)-anchored C1 fragment. During prion diseases, alternative β-cleavage of PrP becomes prominent, producing a GPI-anchored C2 fragment with this particular region intact. It remains unexplored whether physical up-regulation of α-cleavage can inhibit disease progression. The aim of this study is to investigate whether plasmin, a serine protease, induces α-cleavage, and hinders PrPSc propagation.
Materials and Methods: Cleavage of PrP was studied using an in vitro assay composed of recombinant PrP and purified plamin, and in N2a cells incubated with purified plasmin. The functional effect of plasmin in PrPSc propagation was investigated using ScN2a cells and protein misfolding cyclic amplification (PMCA) supplemented with plasmin. The effect of plasmin in prion infectivity was assessed by bioassays using mice infected with aforementioned PMCA products. Detailed experimental conditions were described in Mays et al. (2022).
Results: We characterized the ability of plasmin to perform PrP α-cleavage by showing that plasmin internally cleaves recombinant PrP and generates C1 fragments in a test tube assay and cultured cells. Then, we conducted functional assays using PMCA and prion-infected cell lines to clarify the role of plasmin-mediated α-cleavage during prion propagation. In fact, we demonstrated an inhibitory role of plasmin for PrPSc formation through PrP α-cleavage that increased C1 fragments resulting in reduced prion conversion in PMCA and cell cultures. Furthermore, the reduction of prion infectivity titre in the bioassay of plasmin-treated PMCA material also supported the inhibitory role of plasmin on PrPSc replication.

Conclusions: Our results suggest that plasmin-mediated endoproteolytic cleavage of PrP may be an important event to prevent prion propagation.
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60. The Characteristics of Chinese Human Prion Diseases Based On 20 Years Surveillance Data

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Aims: To describe the characteristics of Chinese human prion diseases based on 20 years Surveillance Data
Materials and Methods: All reporting cases from Jan 2004 to Dec 2023 via China CJD surveillance program were enrolled into this study. The general information, the clinical data, the data of MRI and EEG, and the results of CSF 14-3-3 and PRNP sequencing were carefully collected from the database of national CJD surveillance program. The RT-QuIC experiment became available after 2018. The geography distributions of the patients were calculated based on the provinces they permanently lived. EEG abnormality was recorded only with the presences of periodic sharp wave complexes (PSWC). MRI abnormality was recorded with the presences of high signal in caudate/putamen The interval from onset to diagnosis was calculated from disease onset to disease diagnosis issued by CJD surveillance centre. The survival was evaluated from disease onset to death.
Results: From the national surveillance for human prion diseases in China, about 6000 suspected cases were referred from Jan 2004 to Dec 2023. About 3000 sporadic Creutzfeldt-Jakob disease cases and 291 genetic PrD cases were diagnosed. T188K gCJD, FFI and E200K gCJD are the most common types of mutation. The patients of sCJD distributed all over the country, without seasonal, geography linkage. The incidence peak of sCJD was at the age group of 60–70 year-old. Progressive dementia was most frequently noticed initial symptoms. More than 3/4 of probable sCJD cases were CSF 14-3-3 positive, roughly 2/3 of the cases showed the special abnormalities in MRI and 1/2 of the cases revealed PSWC in EEG. RT-QuIC test has brought help to clinical diagnosis and has been included in diagnostic criteria in China.
Conclusions: 20 years of surveillance data showed the basic characteristics of human prion disease and it still needs long-term and active surveillance in order to have more comprehensive understanding the impact of human prion diseases on public health in China.
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61. An update on surveillance of human prion diseases in Australia

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The Australian National Creutzfeldt-Jakob disease Registry (ANCJDR) has performed nationwide surveillance of all forms of human prion diseases since January 1970, with prospective surveillance occurring from October 1993. This presentation provides an update of the national surveillance figures to 31 December 2023 for all retrospective (to 1970) and prospective (from 1993) cases ascertained.
Materials and Methods: To support surveillance responsibilities, the ANCJDR provides diagnostic platforms for ante- and post-mortem testing for human prion diseases. Post-mortem neuropathological brain examination and prion protein gene (PRNP) assessment are recommended for all suspected CJD cases and is provided by third parties. Annual human prion disease incidence rates are calculated using direct age-standardization, based on Australian Bureau of Statistics estimated resident population data.

Results: In 2023, the ANCJDR received 651 domestic CSF specimens for diagnostic testing of suspected CJD patients. During 2023, 83 persons with suspected human prion disease were formally added to the national CJD surveillance register. The provisional proportion of neuropathology referrals for suspected CJD cases for 2023 is 53%.

For the prospective surveillance period of 1993 to 2023, the annual mean ASMR is 1.47 deaths per million (range 0.7–2.3). Sporadic CJD occurs in Indigenous Australians with a phenotype and incidence rate equivalent to non-Indigenous Australians.

Overall, the vast majority of cases of human prion disease are 'sporadic' (91.5%) while genetic and iatrogenic cases represent 8.5% and < 1%, respectively. In 2023, four genetic cases were confirmed; two additional cases of OPRI and one case of the Q212P mutation were identified through genetic counselling services having been previously misdiagnosed as FTD or psychiatric disorders. Ongoing ANCJDR PrPSc glycotyping has recently uncovered the first confirmed cases of Variably Proteinase Sensitive Proteinopathy (VPSPr) in Australia.

Conclusions: For 2023, the trend of increasing diagnostic CSF referrals and case ascertainment continued, broadly matching the long-term trajectory. Australia continued to be free of vCJD and there were no further cases of iCJD detected. Spatio-temporal clustering of CJD has previously been recognized and was linked to heightened surveillance intensity. The proportion of post-mortem examinations remains high and provides confidence in the Australian surveillance numbers. Accurate diagnosis and subtype classification of prion disease relies on a combination of all diagnostic tests available, including CSF biomarkers, genetic testing, neuropathology, and PrPSc molecular strain typing.

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62. Propagation of distinct CWD prion strains during peripheral and intracerebral challenges of gene-targeted mice

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Aims: Since prion diseases result from infection and neurodegeneration of the central nervous system (CNS), experimental characterizations of prion strain properties customarily rely on the outcomes of intracerebral challenges. However, natural transmission of certain prions, including those causing chronic wasting disease (CWD) in elk and deer, depends on propagation in peripheral host compartments prior to CNS infection. Here we used gene-targeted GtE and GtQ mice, which accurately control cellular elk or deer PrP expression, to assess the impact that peripheral or intracerebral exposures play on CWD prion strain propagation and resulting CNS abnormalities.

Materials and Methods: GtE and GtQ mice were previously generated using a homologous recombination strategy in embryonic stem (ES) cells from FVB mice. In each case, the mouse PrP coding sequence was replaced with the corresponding elk or deer PrP coding sequence. The resulting GtE and GtQ mice are homozygous for the targeted Prnp alleles that express elk and deer PrP respectively. Regulation by Prnp transcriptional control elements results in controlled expression of elk and deer PrPC in GtE and GtQ mice at levels equivalent to that of mouse PrP in wild type mice. Here, we used GtE and GtQ mice to comprehensively assess the impact that peripheral or intracerebral exposures of multiple naturally-occurring cases of North American CWD from elk and deer play on CWD prion strain propagation and resulting CNS abnormalities

Results: Whereas oral and intraperitoneal transmissions produced identical neuropathological outcomes in GtE and GtQ mice and preserved the naturally convergent conformations of elk and deer CWD prions, intracerebral transmissions generated CNS prion strains with divergent biochemical properties in GtE and GtQ mice that were changed compared to their native counterparts. While CWD replication kinetics remained constant during iterative peripheral transmissions and brain titres reflected those found in native hosts, serial intracerebral transmissions produced 10-fold higher prion titres and accelerated incubation times.

Conclusions: Our demonstration that peripherally- and intracerebrally-challenged Gt mice develop dissimilar CNS diseases which result from the propagation of distinct CWD prion strains points to the involvement of tissue-specific cofactors during strain selection in different host compartments. Since peripheral

transmissions preserved the natural features of elk and deer prions whereas intracerebral propagation produced divergent strains, our findings illustrate the importance of experimental characterizations using hosts that not only abrogate species barriers but also accurately recapitulate natural transmission routes of native strains.

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63 Isolation of infectious, oligomeric prions from human and animal prion diseases

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Aim: In Transmissible Spongiform encephalopathies (TSE), or prion diseases, PrPSc fibrils are the target for structural investigation, and recent advances in cryo-EM have elucidated key ultrastructural aspects of prion diseases1. Despite their significance, these structures are limited to rodent-adapted classical scrapie (CS) strains, which represent only a small portion of the structural heterogeneity expected in TSEs. Moreover, a substantial proportion of total infectivity upon fractionation in density gradients has been reported to correlate with oligomers, and non-fibrillar aggregates have been proposed as the most infectious particles2. To explore this further, we purified PrPSc aggregates from brain tissues of humans and animals with different prion diseases and evaluated their biochemical and structural features and role in infectivity.

Material and Methods: Brain homogenates were obtained from a human GSS-A117V case, an atypical scrapie (AS) sheep isolate, and tg338-mice inoculated with AS, all of which characterized by a PrPSc with a N- and C-terminally cleaved PK-resistant core (PrPres), and from voles inoculated with CS, whose PrPres encompasses the whole PrP C-terminus. Samples underwent a purification method3 which yields high levels of purified PrPSc and a modified version involving PK to purify PrPres aggregates4. Purified samples were analysed for their structural characteristics, infectivity, and strain features.

Results: Purified pellets from GSS-A117V, sheep-AS and tg338-AS contained the expected N- and C-terminally cleaved PrPres, although with a PrPres yield lower than that of the vole-adapted CS sample. This lower yield was found to depend on the low density/small size of these aggregates, as they were mainly retained in the supernatant fraction.

Upon inoculation, all purified pellet fractions showed to be highly infectious and fully recapitulate the strain features of the original inoculum.

EM analysis of prions purified from GSS and AS didn't show fibrils, but only small and indistinct non-fibrillar PrPSc particles, whereas fibrils with the expected morphology were recovered from vole-adapted CS.

Conclusions: Our study demonstrates that purified N- and C-terminally cleaved PrPres aggregates are fully infectious and retain all strain features of the original sample, suggesting that the C-terminus of PrPSc can be dispensable for infectivity and strain characteristics. Furthermore, the non-fibrillar morphology of these small prion particles, combined with the high levels of infectivity detected, suggests that oligomers are highly efficient propagators of the disease, and supports the notion that fibrils are not an absolute requirement for prion infectivity.

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References 1. Telling, Nat Commun 20222. Silveira, Nat 20053. Wenborn, Sci Rep 20154. Vanni, Brain 2020

64. Translating structural biology into rationally-designed vaccines for neurodegenerative diseases

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Aims: Protein misfolding causes neurodegenerative diseases such as Alzheimer's, Parkinson's, or the prion diseases by adopting specific, beta-sheet rich conformations. Numerous structures of disease-causing, misfolded proteins have been elucidated using cryo electron microscopy and other techniques. Insights from these structures can be used to rationally design vaccines targeting specific epitopes that are present on the surface of the misfolded proteins, but not their natively folded precursors. The efficacy of these vaccines can be tested in transgenic animal models recapitulating specific disease phenotypes.
Materials and Methods: Published protein structures and molecular models were used to select discontinuous, structured epitopes from the surface of the disease-causing confirmations of the amyloid beta peptide, tau protein, alpha-synuclein, or prion protein. The selected epitopes were then inserted into innocuous scaffold proteins that adopt a beta-sheet rich structure. The resulting vaccine candidates were expressed in E. coli, purified, in vitro refolded, and subjected to rigorous quality controls to ascertain proper folding. Transgenic mouse models for the respective neurodegenerative disease were then immunized using a prime-boost schedule and followed for extended periods of time. At a given time, mouse brains were collected and analysed for the accumulation of misfolded proteins to determine vaccine efficacy.
Results: For example, 5xFAD mice were engineered to start accumulating amyloid beta plaques by two months of age and have widespread amyloid beta plaque depositions by six months of age. 5xFAD mice that were immunized with our engineered, amyloid beta-targeting vaccines developed immune responses capable of recognizing the misfolded amyloid beta that was used to design the vaccines, demonstrating the specificity of the selected surface epitopes and of the resulting engineered vaccines. Male 5xFAD mice that were immunized after the onset of plaque formation were found to be free from amyloid beta plaques by ~200 days of age, while immunized female 5xFAD mice had only a ~50% reduction in plaque load compared to unimmunized controls. More work will be needed to determine the factors that cause this sex-dependent difference in vaccine efficacy and to increase efficacy for female mice.
Conclusions: Translating insights from the structural biology of misfolded proteins allows to generate disease-specific, engineered vaccines capable of protecting transgenic animal models of neurodegenerative diseases. Active immunotherapy can achieve prophylactic or even therapeutic efficacy in relevant transgenic mouse models. Extended clinical trials would be needed to determine the vaccines' potential to prevent or treat neurodegenerative diseases in humans.
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65. VPS35-deficiency contributes to Alzheimer's disease development

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Aims: Our long-term research aims are to enhance understanding of the communications among different brain cells (neurons, astrocytes, and microglia) and how disruption of their communication contributes to the development and progression of Alzheimer's disease (AD)
Materials and Methods: A combination of in vitro and in vivo (mouse models) methods is used, which includes molecular, cell biological, electrophysiological, immunological, and behaviour tests.
Results: Vps35-loss in different types of brain cells, including neurons and glial cells, contributes to various types of neurodegenerative diseases, including AD and related dementias.
Conclusions: Our results enhance our understanding the communication among different brain cells, and

insights the potential to reveal new therapeutic opportunities for neurodegenerative disorders.

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66. Peptide-addressed liposome -embedded therapeutic systems: PALETS delivering tools to alter protein expression and limit pathologic protein misfolding

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Aims: Prion diseases are members of neurodegenerative, protein misfolding diseases (NPMDs) that include Alzheimer's, Parkinson's and Huntington diseases, Amyotrophic Lateral Sclerosis, tauopathies, traumatic brain injuries, and chronic traumatic encephalopathies. No known therapeutics improve quality of life or survival times of humans afflicted with prion disease. We and others developed a new approach to NPMD therapy based on reducing the amount of the normal, host-encoded protein that acts as a substrate for misfolding into pathologic forms using RNA interference, a pathway that decreases levels of mRNA encoding a particular protein. We developed a non-invasive therapeutic delivery system consisting of liposomes complexed with a peptide that delivers the nanoparticles across the blood-brain barrier to Acetylcholine-expressing cells. These peptide-addressed, liposome-embedded therapeutic systems (PALETS) deliver therapeutic cargo to the central nervous system. We used PALETS to deliver PrP siRNA to neuronal cells to decrease expression of the normal cellular prion protein, PrPC, which acts as a substrate for prion replication.

Materials and Methods: Liposomes were incubated with control or PrP siRNA for 10 min, then RVG-9 r or control RVM peptide for 10 min in a 10:1:10 L:S:P ratio. We assessed PALET pharmacokinetics and pharmacodynamics by live animal imaging and flow cytometry. Mice were infected intraperitoneally or intracranially with 103 LD50 units of RML 5.0 prions. We treated mice intravenously and/or intranasally with siRNA PALETS 3–9X and assessed for clinical prion disease by survival time, biochemistry, neuropathology and associated behavioural deficits by burrowing and nesting tests. We assessed immune responses to PALETS by IgG enzyme-linked immunosorbent assay.

Results: We show that PALETS efficiently deliver PrP siRNA to 47% of brain cells, decrease PrP expression 70% and extends survival and improve behaviour of prion-infected mice. However, repeated immunization elicited a strong IgG antibody response to the delivery peptide.

Conclusions: PrP siRNA PALETS extend survival time up to 22% in mice that remain immunotolerant to treatment. After three immunizations, mice can develop a strong antibody response to the delivery peptide. PALETS hold promise as efficient delivery systems that can be sized to carry therapeutic cargo of interest, such as Cas9 and derivatives, without requiring truncated or spliced versions that greatly reduce efficiency, like adeno-associated viral vectors, for example. PALETS offer a flexible, non-viral therapeutic delivery system for a single dose somatic cell genome editing approach for treating monogenic protein misfolding diseases.

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67. Skin misfolded protein-seeding activities as diagnostic biomarkers for neurodegenerative diseases

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Aims: To summarize and highlight the recent development of exploring the seeding activities of various skin misfolded proteins as diagnostic biomarkers across neurodegenerative diseases

Materials and Methods: Autopsy and biopsy skin samples were obtained from prion diseases (PrDs), Parkinson's disease (PD) and other synucleinopathies, as well as Alzheimer's disease (AD) and other tauopathies diagnosed neuropathologically or clinically, respectively. The seeding activities of various skin misfolded proteins were detected by real-time quaking-induced conversion (RT-QuIC) and protein misfolding cyclic amplification (PMCA) assays. The substrates used in RT-QuIC and PMCA assays were their normal recombinant proteins purified from E. coli expressing hamster PrP90-231, human wild-type α-Synuclein (αSyn), or human tau fragments (3RCF and 4 RCF), respectively.

Results: We were the first to detect infectious prions by both seed-amplification assay with RT-QuIC and animal transmission study in the skin tissue of patients with sporadic Creutzfeldt-Jakob disease (sCJD), the most common form of human PrDs. Furthermore, we have demonstrated that skin prions can be detected much earlier than brain damage that occurs in animal models, suggesting their potential as a preclinical diagnostic biomarker for prion diseases. Our research endeavours have expanded to include investigations into PD and AD, demonstrating the robust sensitivity and specificity of the RT-QuIC assay in detecting the seeding activity of αSyn in both autopsy and biopsy skin tissues obtained from patients with PD. We have also set up our study on tau RT-QuIC assays with brain tissues of AD and non-AD individuals confirmed neuropathologically, followed by our recent investigation that has finalized the tau RT-QuIC assay of autopsy and biopsy skin samples obtained from individuals with tauopathies including AD.

Conclusions: Our serial studies suggested that the seeding activities of various skin misfolded proteins can be biomarkers for diagnoses of neurodegenerative diseases including PrDs, PD and AD.

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68. The Expanding Prion Paradigm

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The discovery of prions explained how a single pathogen can cause one disease process but present as three different illnesses: (1) infectious, (2) genetic, or (3) sporadic. Prions are unique in that a wild-type prion protein can occur as an infectious or sporadic illness, while mutant prion proteins manifest as inherited maladies. Recent studies argue that amyloid-β (Aβ) prions, tau prions, and α- synuclein prions are three chemically distinct prions, all of which can adopt pathogenic, self-propagating conformations. Aβ prions cause Alzheimer's disease (AD) while α-synuclein prions cause Parkinson's disease (PD). In light of these advances, it would be prudent to abandon the quest for an alternate word to supplant prion and to discard terms such as prion-like, which denotes a level of uncertainty that is no longer justified. Expending effort to devise an alternative term to replace the word 'prion' would seem to be a futile exercise. More important is the discovery of efficacious drugs that block the formation of prions. Currently, more than 40 million people on our planet suffer from the ravages of AD and 10 million suffer from PD. As we look to the future, the projected increase in the number of centenarians will lead to a flood of additional patients with AD and PD by 2050. Thus, it is critical that we discover more effective drugs to treat prion diseases; however, even more important, we need to prevent these devastating central nervous system prion disorders. Can we discover inexpensive methods for the early detection of prion disorders and create highlyeffective drugs to prevent these illnesses? While the challenges presented by prion diseases are substantial, we already have some of the new tools needed to attack these

formidable disorders. Most important, we need to discover (1) highly effective drugs that prevent AD and (2) other pharmaceuticals that prevent or effectively slow PD.

69. 14-3-3 as a useful biomarker in the process of developing the method of real-time quaking induced conversion (RT-QuIC) for prion disease identification

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Abstract: With the ageing of population, rapid and progressive dementia (RPD) is not uncommon anymore in hospitals in China. Among the RPDs one unique category of the diseases are prion diseases, which should be identified due to their significance in public health and poor outcome in treatment. In the past 10 years an important laboratory testing method in prion disease diagnosis, real-time quaking induced conversion (RT-QuIC), has been developed. This test has been proved very sensitive and specific, and can be used pre-mortem with CSF instead of brain tissue post-mortem. In developing this RT-QuIC assay here in China, one difficulty we faced was that there were no neuro-pathological proof (definite diagnosis based on the international guidelines of prion disease diagnosis) on the patients, from whom we obtained the related CSF. What we did was to use 14-3-3 (a traditional laboratory parameter based on the guidelines for a probable prion disease diagnosis) as a screening biomarker to select certain CSF samples, and run the samples repeatedly to optimize the testing condition and check the reproducibility and consistency. A total of 14 samples were selected first (seven 14-3-3 positive and negative separately), and 6 repeats of the experiments were performed each week on each sample (triplicate wells 3 times and quadruplicate wells 3 times). Thirteen out of 14 samples showed 100% reproducibility. After we got confident about the method, we validated the method by testing more clinical samples and communicating with clinicians to make sure the result makes sense in matching other clinical proof (clinical evaluation, EEG, brain imaging, etc.). A total of 45 samples have been tested so far, and some representative cases will be presented to indicate that 14-3-3 is still a decent bio-marker in quick screening prion diseases before RT-QuIC result can be obtained.

Short Bio of Keding Cheng: Keding Cheng is a professor of KingMed College of Laboratory Medicine of Guangzhou Medical University. He has been trained as a medical doctor but has done a lot of research work in prion disease laboratory method development. One method is called end-point quaking induced conversion (EP-QuIC), an improved method of RT-QuIC, for sCJD identification. Dr Cheng has published many papers in applications of new technologies for clinical laboratory diagnosis, and his main interest now is to develop more easy-to-use, blood-based diagnostic method on more commonly-seen neurodegenerative diseases (AD, PC, ALS, etc.) besides prion diseases.

70 Molecular Mechanism of Strain-distinct α-Synuclein and Tau Cross-seeding Uncovered by Correlative Approach with O-PTIR Super-resolution Imaging

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Aims: Co-occurrence of amyloid proteins such as α-synuclein (αSyn) and Tau in synucleinopathies and tauopathies implies a complicated interplay between the two proteins. In vitro studies have shown that cross-seeding of αSyn and Tau synergistically enhances their fibrillization, forming various amyloid strains enriched with β-sheets. In the present study we aim to further determine the underlying molecular mechanism of the amyloid-specific structures of various strains of amyloid proteins.

Materials and Methods: Super-resolution optical photothermal infrared (O-PTIR) microspectroscopy combined with confocal microscopy provides a unique opportunity to image the structure-sensitive information on protein-

specific macromolecular assemblies in situ. Using this correlative imaging approach, we have characterized structural rearrangements of newly formed intracellular αSyn inclusions cross-seeded by preformed fibrils (PFFs) made of αSyn and Tau.

Results: We found that Tau (Tau3R) mixed or co-fibrilized with αSyn contain the most abundant β-sheets and displayed higher seeding potency, which also leads to stronger αSyn and Tau phosphorylation in newly formed αSyn inclusions. Importantly, we demonstrate that these inclusions inherited structural motifs of the donor seeds and are characterized by different spatial and structural evolution. Using correlative approach for structural imaging of amyloid proteins directly in cells at submicron resolution, the present study revealed the underlying molecular mechanisms of amyloid formation.

Conclusions: We revealed that αSyn inclusions retain the conformational characteristics reminiscent of the templating mixed and hybrid αSyn/Tau seeds and justified the unique application of the super-resolution optical photothermal infrared microscopy for structural imaging of macromolecular assemblies.

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71. SIRT1 regulates mitochondrial damage in N2a cells treated with the prion protein fragment 106–126 via PGC-1α -TFAM – mediated mitochondrial biogenesis

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Aims: Mitochondrial damage is an early and key marker of neuronal damage in prion diseases. As a process involved in mitochondrial quality control, mitochondrial biogenesis regulates mitochondrial homoeostasis in neurons and promotes neuron health by increasing the number of effective mitochondria in the cytoplasm. Sirtuin 1 (SIRT1) is an NAD+-dependent deacetylase that regulates neuronal mitochondrial biogenesis and quality control in neurodegenerative diseases via deacetylation of a variety of substrates. In previous studies, whether mitochondrial biogenesis is impaired in prion disease and the role of SIRT1 in mitochondrial biogenesis have not been fully studied. The aim of this study is to investigate the functional role of and regulatory mechanisms enacted by SIRT1 in mitochondrial biogenesis during prion disease.

Materials and Methods: We constructed a prion disease cell model induced by PrP106-126, and tested SIRT1 level and mitochondrial biogenesis of this model. To demonstrate the underlying mechanism regarding SIRT1 regulation of mitochondrial biogenesis at the transcriptional and translational levels, we overexpressed and knocked down SIRT1, or activated and inhibited the activity of SIRT1 in this prion disease cell model.

Results: We found that both SIRT1 protein levels and deacetylase activity decreased, and SIRT1 overexpression and activation significantly ameliorated mitochondrial morphological damage and dysfunction caused by the neurotoxic peptide PrP106-126. Moreover, we found that mitochondrial biogenesis was impaired in prion diseases, and SIRT1 overexpression and activation alleviated PrP106-126-induced impairment of mitochondrial biogenesis in N2a cells. Further studies in PrP106-126-treated N2a cells revealed that SIRT1 regulates mitochondrial biogenesis through the PGC-1α-TFAM pathway. Finally, we showed that resveratrol resolved PrP106-126-induced mitochondrial dysfunction and neuronal apoptosis by promoting mitochondrial biogenesis through activation of the SIRT1-dependent PGC-1α/TFAM signalling pathway in N2a cells.

Conclusions: Taken together, our findings further describe SIRT1 regulation of mitochondrial biogenesis and improve our understanding of mitochondria-related pathogenesis in prion diseases. Our findings support further investigation of SIRT1 as a potential target for therapeutic intervention of prion diseases.

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72. Prion disease features in Japan based on the national surveillance from 1999 to 2024

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Aims: To elucidate epidemiological features of prion disease (PrD) in Japan during last 25 years.
Materials and Methods: Nationwide surveillance has been conducted by Prion Disease Surveillance Committee in Japan since 1999. The committee was composed of 10 district members and specialists in epidemiology, neurosurgery, genetics, EEG, neuroimaging, CSF biomarkers, neuropathology, and genetic counselling. The committee members review each case record form of suspected PrD in Japan and provide the accurate diagnosis of the case based on the clinical and laboratory information twice a year.
Results: Information of 9761 suspected PrD patients was obtained and until February 2024. There were 5055 PrD cases [sporadic CJD; 3852 cases, variant CJD;1, dura matter-associated CJD (dCJD); 99, and genetic PrD;1124 (genetic CJD 927, Gerstmann-Sträussler-Scheinker disease; 189, and fatal familial insomnia; 8). The annual incidence increased from 0.7 to 2.3 per million from 1999 to 2015.

As to codon 129 status of sCJD, 62% is MM, 3.0% is MV, 0.3% is VV, and 35% is undetermined.

For gCJD cases, the most common mutation is V180I (54.7%) followed by M232R (11.5%) and E200K (11.3%) and others.
Conclusions: Our surveillance confirmed the increase of PrD incidence and characteristic features of PrDs in Japan. Continuous surveillance of PrDs is very important and has contributed greatly to various types of research to overcome PrDs.
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73. Conformational change of α-synuclein fibrils in cerebrospinal fluid from different clinical phases of Parkinson's disease

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Aims: α-Synuclein (α-syn) fibrillar aggregates are a major component of Lewy bodies and Lewy neurites presenting as the pathology hallmark of Parkinson's disease (PD). Studies have shown that α-syn is potential to form various conformational fibrils associated with different synucleinopathies, but whether the conformation of α-syn fibrils would change during the process of disease progression is unclear.
Materials and Methods: We amplified α-syn aggregates from the cerebrospinal fluid (CSF) of PD patients staged in different clinical phases, including a preclinical PD (pre-PD), four middle-to-late staged PD (mid-PD) and a late staged deceased PD (late-PD) patients. The high-resolution structures of the CSF-amplified fibrils were determined by using cryo-electron microscopy (cryo-EM). We also examined the pathology of the CSF-amplified α-syn fibrils in rat primary neurons.

Results: The α-syn fibrils derived from the late-PD patient are most potent in inducing endogenous α-syn aggregation in primary neurons, followed by the four mid-PD fibrils, while the pre-PD fibrils exhibit the lowest activity. Furthermore, their structures exhibit remarkable differences in a minor but significant population of conformational species in each fibril sample, which in the pre-PD fibrils appears morphologically straight, in the mid-PD fibrils is composed of two intertwining protofilaments, while in the late-PD fibrils represents a single protofilament formed by a distinctive conformation of α-syn.

Conclusions: Our work demonstrates structural and pathological differences between α-syn fibrils derived from PD patients at a spectrum of clinical stages, which suggests potential conformational transition of α-syn fibrils during the progression of PD

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Acknowledgement: D.L., J. W. and C.L. designed the project. Y.F. prepared the patients-derived fibril samples, performed the biochemical and cellular assays. Y. S. and Y.F. performed the cryo-EM experiments. Y. S. built and refined the structure model. W.X., Q. Z. and Q.C. assisted in cryo-EM data collection and processing as well as model building. Y.S. and Y. T. assisted in figures preparation. W.Y., Y.L., Y. T. contributed to the clinical assessments of PD patients. Y.S., F.L. and J. W. contributed the CSF samples collection. All the authors are involved in analysing the data and contributing to manuscript discussion and editing. Y.F and D.L. wrote the manuscript.

74. Different Classical Scrapie and CWD Strains Circulating in Europe as Shown by Prion Strain Typing Using Ovinized Mice (Tgshp IX)

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Aims: Transmissible spongiform encephalopathies (TSEs) comprise several prion diseases and are caused by the conversion of the host-encoded prion-protein (PrPC) into a pathologic isoform (PrPSc). PrPSc can take on numerous conformations commonly referred to as 'prion strains'. The most important TSEs in animals are classical and atypical scrapie in small ruminants, Chronic Wasting Disease (CWD) in cervids and bovine spongiform encephalopathy (BSE) in cattle. While classical BSE occurs as a single strain, that easily overcomes the transmission barrier to even distant species, including humans, classical scrapie and CWD are polymorphic, displaying various prion strains with uncertain abilities to cross transmission barriers. Therefore, a thorough characterization of scrapie and CWD isolates will be sought using an ovinized mouse model and classical BSE as reference.

Materials and Methods: European field goat scrapie isolates and a panel of Canadian and European CWD field isolates were inoculated intracerebrally into the Tgshp IX mouse model, which overexpresses the ovine ARQ-genotype. Mice brains were analysed using biochemical, histopathological and immunohistochemical methods to define the strain phenotype and with respect to the CWD isolates, evaluate their potential to overcome the ovine transmission barrier.

Results: As expected, the transmission barrier of the European goat TSE isolates to the Tgshp IX model can be considered very low and allowed a clear-cut discrimination of atypical scrapie, the CH1641 strain and goat BSE as well as the identification of three classical scrapie strains. The classical scrapie strains further revealed geographical clusters and indicated the existence of sub-strains that slightly resemble either classical BSE or the CH1641 strain. In contrast, most CWD isolates hardly crossed the ovine transmission barrier. However, isolates of Canadian elk, moose and red deer as well as two Swedish moose isolates displayed low attack rates after first passage. In particular the comparison of the Swedish moose isolates provided evidence for prion strain variability even after first passage. Preliminary results of Norwegian red deer and reindeer isolates propose strain adaptation after second passage.

Conclusions: The Tgshp IX mouse model proved to be of great value for prion strain characterization and the evaluation of potential interspecies transmission between wildlife, livestock and humans: This study not only showed that different classical scrapie strains circulate in European goat populations, but also revealed the occurrence of sub-strains within given

isolates. Moreover, the investigation of the zoonotic potential of European and Canadian CWD isolates, indicates that CWD might pose a threat to sheep, once shed into the environment.

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75. Discovery of Two Compounds from Moringa oleifera that Modulate Prion Protein Aggregation

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Aims: Prion diseases, or transmissible spongiform encephalopathies (TSEs), are caused by the conversion of the monomeric cellular prion protein (PrPC) into scrapie prion protein (PrPSc), leading to the formation of toxic aggregates that increase exponentially as the disease progresses. These diseases are fatal and incurable, with no preventive treatments currently available. Therefore, searching for new compounds with antiprion activity, capable of preventing prion conversion and aggregation, is of significant therapeutic interest. In this context, the plant Moringa oleifera Lamark (Moringaceae) is a medicinal plant with various pharmacological properties already described in the literature, including hypotensive, hypocholesterolemic, antioxidant, and anti-herpes simplex virus type 1 activity, as well as an antiproliferative effect significant for cancer prevention. Thus, this study aims to evaluate the antiprion effect of Moringaceae.
Materials and Methods: For this purpose, we prepared different hydroalcoholic extracts from this plant's leaf and flower and assessed their antiprion effect using the RT-QuIC assay. This assay can evaluate the interference of therapeutic candidates in the aggregation kinetics of PrPC based on the ability of PrPSc to self-replicate. Affinity selection-mass spectrometry (AS-MS) and microfractionation were used to isolate and identify compounds with more than 50% inhibition of aggregation.
Results: Our results showed that the extracts exhibited a significant inhibitory effect on aggregate formation in a dose-dependent manner. Leaf extracts were more potent than flower extracts, with differences observed between the types of extractions performed. The MoLV-EHI extract underwent microfractionation, which allowed for identifying two active molecules responsible for the antiprion effect. Additionally, both the extracts and the isolated molecules reduced the presence of aggregates even when added after their formation.
Conclusions: Therefore, we can conclude that Moringaceae extracts contain molecules that exhibit antiprion activity in vitro, inhibiting prion conversion and aggregation and also displaying disaggregase activity. Identifying the molecules in these extracts responsible for this activity allows for evaluating the mechanism and their antiprion potential using other experimental approaches, making these extracts attractive in searching for effective therapies for prion diseases.

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76. *De novo* generation of α-synuclein aggregates in normal mouse brain tissue sections: evidence for the presence of silent transmissible protein seeds in the normal brain

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Aims: The aggregation and accumulation of α-synuclein (αSyn) in the brain are key molecular features of Parkinson's disease (PD), yet whether there are silent αSyn seeds in the normal brain remain unclear. The aim of our current study was to investigate the hypothesis that a small quantity of silent misfolded αSyn seeds exists naturally in the brains of healthy individuals.

Materials and Methods: Normal C57 mouse brain tissue sections were incubated with an amplification buffer containing excess recombinant mouse αSyn (rMαSyn) to screen for misfolded αSyn seeds. As controls, recombinant human αSyn, prion protein (PrP)90–231, and 4R- and 3R-tau were also tested. The end-products of our amplification assay (AA) were analysed for misfolded αSyn formation using proteinase K (PK) digestion followed by western blotting, immunofluorescence (IF), immunohistochemistry (IHC) with various anti-αSyn antibodies, Congo Red staining, and transmission electron microscopy (TEM). To confirm the seeding activity of the AA end-products, we employed real-time quaking-induced conversion (RT-QuIC), along with transmission studies using organoid and cell cultures, and in vivo experiments involving intracranial injection of AA end-products into C57 mice.

Results: Our IF microscopy revealed intense thioflavin T (ThT) fluorescence in brain tissue sections treated with our amplification assay (AA), but not in untreated sections, renal tissue sections, or sections treated with other substrates like recombinant human αSyn, hamster PrP90-231, or 4R- and 3R-tau. After treatment of mouse brain tissue sections with PK at 50 μg/mL at 37°C for 30 min, the ThT signal was significantly decreased compared to that without PK-treatment. Congo Red staining confirmed the presence of protein aggregates. IHC using the anti-αSyn antibody MJFR14 verified that the ThT-positive structures were primarily composed of αSyn. We also found that preformed αSyn fibrils (PFF) were efficiently amplified by our AA. Currently, we are examining the seeding activity of the AA end-products using RT-QuIC, organoid and cell models, as well as animal models.

Conclusions: Our study has, for the first time, demonstrated the de novo generation of αSyn aggregates in normal mouse brain tissue sections. This provides proof-of-concept evidence for the existence of silent transmissible protein seeds in the healthy brain.

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