



Prion and Prion-like Diseases in Humans

To cite this article: (2014) Prion and Prion-like Diseases in Humans, Prion, 8:sup1, 110-139, DOI: [10.4161/pri.29371](https://doi.org/10.4161/pri.29371)

To link to this article: <https://doi.org/10.4161/pri.29371>



Copyright © 2014 Landes Bioscience



Published online: 01 Apr 2014.



Submit your article to this journal [↗](#)



Article views: 1052



View related articles [↗](#)



View Crossmark data [↗](#)

Prion and Prion-like Diseases in Humans

P.179: Sporadic Creutzfeldt-Jakob disease in Canada

Zheng Wang,¹ Gerard Jansen,^{1,2} Stacy Sabourin,¹
Rolande D'Amour,¹ Tim Connolly,¹ Jennifer Kruse,¹
David J Knox,³ Neil R Cashman,⁴ and Michael B Coulthart¹

¹The Canadian Creutzfeldt-Jakob Disease Surveillance System; Public Health Agency of Canada; Ottawa, ON Canada; ²Department of Pathology; Ottawa Hospital; Ottawa, ON Canada; ³National Microbiology Laboratory; Public Health Agency of Canada; Winnipeg, MB Canada; ⁴Brain Research Centre; University of British Columbia; Vancouver, BC Canada

Background. Sporadic Creutzfeldt-Jakob Disease (sCJD) is a fatal, transmissible neurodegenerative disease. Systematic surveillance has repeatedly shown annual mortality in the range 1 to 2 per million population, has elucidated key characteristics of sCJD, and led to recognition of a new form of CJD, variant CJD (vCJD), which is associated with BSE. In 1998, Canada launched comprehensive national CJD surveillance to assess the characteristics of CJD in Canada, identify any acquired cases of CJD (such as vCJD, of which 2 imported cases have been identified in Canada to date), and mitigate public health risks. This study describes the epidemiology of sCJD in Canada from 1998 to 2012.

Methods. Case ascertainment was based on internationally accepted criteria. Demographic and medical information were collected by standardized questionnaire and medical chart review. Poisson regression and descriptive analysis were employed.

Results. A total of 563 sCJD deaths (definite: 462, probable: 101) in Canadian residents were registered from 1998 to 2012. Average annual sCJD mortality was 1.2 per million population, increasing gradually from 0.9 in 1999 to 1.7 in 2012 ($P = 0.0004$). All provinces saw average annual mortalities ranging from 1.0 to 1.6 ($P = 0.25$), except three territories where population is small (~25,000 to ~45,000) and no cases were identified. sCJD occurred at similar rates in males (1.1) and females (1.2) ($P = 0.21$). sCJD was rare under 50 years of age with only 11 cases identified (2.7%). Mortality increased after 50 and peaked at 7.4 per million in the 70–74 age group. Median age at death was 69 and median duration of illness was 4 months. Genotype at codon 129 ($N = 358$) revealed that the MM subgroup accounted for 223 (62%, median age at death: 69, duration: 4), the MV subgroup was 82 (23%, median age at death: 68, duration: 9), and the VV subgroup was 53 (23%, median age at death: 66, duration: 5). Results of molecular typing (Parchi Scheme) for 256 cases are: MM1: 140, MM2: 11, MV1: 28, MV2: 18, VV1: 5, VV2: 25, Mixture: 29.

Conclusion. Characteristics of sCJD in Canada are consistent with those observed in other countries. The increase in sCJD mortality can be partly attributed to increased awareness of CJD among Canadian clinicians. These findings support the conclusion that Canadian CJD surveillance system is sufficiently sensitive to accurately characterize the epidemiology of sCJD in Canada, and to detect potential additional cases of acquired CJD such as vCJD or human chronic wasting disease.

P.180: Performances of various brain-derived spikes commonly used to evaluate prion removal devices

Nathalie Lescoutra-Etchegaray,¹ Audrey Culeux,¹
Valérie Durand,² Chrystlain Sumian,³ Jean-Philippe Deslys,²
and Emmanuel E Comoy²

¹Macopharma; Fontenay-aux-Roses, France; ²CEA, Institute of Emerging Diseases and Innovative Therapies (iMETI); Division of Prions and Related Diseases (SEPIA); Fontenay-aux-Roses, France; ³Macopharma; Tourcoing, France

Background. In a context of uncertainties on vCJD transmission through blood transfusion, several processes and devices have been developed to remove prions from labile or stable blood-derived products, including the resin affinity-based P-Capt filter. The assessment of the performances of these devices or processes with infectious blood is limited by the low level of blood-borne infectivity. To date, several brain spike preparations based on sonication and/or centrifugation steps are used but their relevance to mime blood-borne infectivity remain discussed. We aimed to compare the ability of an affinity resin to retain prion endogenous or exogenous (issued from different spike preparations) blood infectivity.

Study design and methods. Plasma from 263K-infected hamsters and plasmas spiked with different brain homogenates preparations were filtered through the affinity resin packed in columns. After filtration, resins were recovered and intracerebrally injected into hamsters. Incubation periods of these animals were analysed and compared.

Findings. Our study confirms the ability of this resin to retain both endogenous plasmatic and exogenous brain-derived infectivity in the 263K-infected hamster model. Surprisingly, the different brain-derived spikes induced similar survival curves, but with shorter incubation periods than those obtained with blood-derived products. Our results suggest that blood infectivity is hardly mimicked by brain extracts, and confirm that endogenous infectivity study in an appropriate animal model for human prion disease is compulsory to definitely assess the efficiency of blood prion removal.

P.181: Involvement of lysosome-associated degradation in fulminant neurodegeneration

Julien Donnelier, Samuel T Braun, and Janice EA Braun

Hotchkiss Brain Institute; Department of Physiology and Pharmacology; University of Calgary; Calgary, AB Canada

The misfolding, aggregation and accumulation of proteins is known to be the causal event in many neurodegenerative disorders. Under normal conditions a network of chaperones and proteolytic systems fight against the toxic effects of aberrant proteins. Multiple lines of evidence indicate that an imbalance between the production and clearance of proteins initiates a toxic sequence of events leading to protein misfolding, synaptic dysfunction, synaptic loss and neurodegeneration. Lysosomes are highly acidic organelles that contain hydrolytic enzymes that receive and clear macromolecules from the secretory, endocytotic and autophagic pathways. The link between synaptic loss and lysosomal function is an area that is not well investigated. To begin to assess the role of lysosome function in neurodegeneration, we used mice lacking the chaperone cysteine string protein (CSP α) as a model of neurodegeneration. CSP α deficient mice are normal at birth, but postnatally develop an impairment of synaptic function in an activity-dependent manner followed by a fulminant form of neurodegeneration, paralysis and early death. CSP α -null mice were injected intraperitoneally with either 5 mg/kg chloroquine, a weak base that increases lysosomal pH and inhibits lysosomal activity or 20 mg/kg Z-Phe-Ala-diazomethylketone, which increases lysosomal activity by increasing lysosomal cathepsin B levels, or vehicle (saline/DMSO) every 2 days beginning at post-natal day 5 for 2 weeks and weight loss was monitored. Following treatment, synaptic fractions were isolated to determine the production and clearance of amyloid precursor protein and A β_{1-42} , a 42 residue long β amyloid protein as well as synaptic plasma membrane/lysosome markers. Our preliminary results indicate that Z-Phe-Ala-diazomethylketone slows the onset of neurodegeneration. This line of investigation will directly answer the question whether the progression of neurodegenerative disorders is altered by pharmaceutical agents that up-regulate or down-regulate lysosomal activity.

P.182: PrP-A β binding as a quality control for ADDL preparations

Daniel Wright, Silvia A Purro, Andrew J Nicoll, and John Collinge

MRC Prion Unit and Department of Neurodegenerative Disease; UCL Institute of Neurology; London, UK

Prefibrillar oligomeric species of amyloid-beta (A β) are thought to play an important role in the pathogenesis of Alzheimer's disease (AD), possibly acting via one or more receptors. Indeed, the cellular form of the prion protein (PrP^C) has

been shown to bind tightly to amyloid-derived diffusible ligands (ADDLs)—synthetic preparations of A β oligomers that mimic the toxicity displayed by AD brain-associated species—in both recombinant PrP-based assays as well as human and murine cell models. Many different protocols for the preparation of ADDLs have been described that vary in their methods of solubilisation, incubation and harvesting. Using Dissociation-Enhanced Lanthanide Fluorescent Immunoassay (DELFI) as a readout for PrP-A β binding, we show that synaptotoxic ADDL preparations bind with low nanomolar affinity to plate-bound, recombinant human PrP, and that the affinity of binding varies according to the volume of DMSO used to resolubilise A β . Indeed, we found that ADDLs prepared in 0.1% DMSO displayed between 5- and 10-fold lower affinity for PrP than ADDLs prepared in 2% DMSO, suggesting the extent to which A β is resolubilised significantly affects the oligomer content of preparations. Consistent with this, we found that poorly resolubilised ADDL preparations displayed markedly reduced binding to murine hippocampal cultures compared to fully resolubilised preparations. Moreover, we show for the first time that the same ADDL preparation displays similarly high affinity for both human and murine forms of recombinant PrP. Thus, by exploiting its affinity for A β oligomers, we describe a standardised procedure by which PrP can be effectively used to routinely quality control different ADDL preparations.

P.183: Multi-parameter MRI analysis in the national prion monitoring cohort

Marie-Claire Porter,^{1,2} Enrico De Vita,³ Ivor Simpson,⁴ Sebastien Ourselin,⁴ Jessica Lowe,^{1,2} Edit Franko,^{1,2} Nina Bjurstrom,^{1,2} Peter Rudge,^{1,2} Simon Mead,^{1,2} John Collinge,^{1,2} John Thornton,³ and Harpreet Hyare^{1,2}

¹NHS National Prion Clinic, National Hospital for Neurology and Neurosurgery; London, UK; ²MRC Prion Unit; Department of Neurodegenerative Disease; UCL Institute of Neurology; London, UK; ³Academic Neuroradiological Unit; Department of Brain Repair and Rehabilitation; UCL Institute of Neurology; London, UK; ⁴UCL Centre for Medical Image Computing; London, UK

Quantitative MRI measures can detect the presence of micro-structural pathology in human prion disease that is not visible on conventional MR imaging. Few studies have examined longitudinal change and correlation with clinical measures. To test if longitudinal change could be identified on quantitative MRI, we performed a multiple-parameter analysis in the largest data set of patients with prion disease to date.

Multiple-parameter analysis of quantitative MRI sequences including diffusion tensor imaging, magnetisation transfer ratio and T2-weighted imaging was performed in 101 subjects (30 asymptomatic gene mutation carriers, 37 symptomatic patients and 34 healthy controls). The analysis included voxel-based morphometry, voxel-based analysis of diffusion tensor imaging and magnetisation transfer imaging measures, grey and white matter histograms and region of interest analysis.

Symptomatic patients showed significant longitudinal grey matter atrophy. Additionally a significant decrease in magnetisation transfer ratio and mean diffusivity was observed in grey matter structures; the decline in all MRI parameters strongly correlated with a decline in a functionally orientated outcome measure, the MRC Prion Disease Rating Scale. These data show that multi-parameter MRI analysis can identify longitudinal microstructural grey matter changes and may be able to provide objective and anatomically-specific biomarkers of disease progression in future clinical trials.

P.184: Human prion diseases in Japan: a prospective surveillance from 1999

Nobuo Sanjo,^{1,2} Maya Higuma,¹ Masaki Hizume,¹

Fumiko Furukawa,¹ Yosikazu Nakamura,²

Tetsuyuki Kitamoto,² Masahito Yamada,² Kenji Sakai,²

Ichiro Nozaki,² Moeko Noguchi-Shinohara,²

Tsuyoshi Hamaguchi,² Fumio Moriwaka,² Masashi Aoki,²

Fumiaki Tanaka,² Masatoyo Nishizawa,² Masatoshi Takeda,²

Takashi Inuzuka,² Koji Abe,² Hiroyuki Murai,²

Shigeo Murayama,² Masaki Takao,² Katsuya Satoh,²

Masafumi Harada,² Nobuhito Saito,² Ichirou Takumi,²

and Hidehiro Mizusawa,^{1,2}

¹Department of Neurology and Neurological Science; Tokyo Medical and Dental University; Graduate School of Medical and Dental Sciences; Tokyo, Japan; ² Prion disease Surveillance Committee; Tokyo, Japan

Introduction. A nationwide surveillance system for human prion diseases (PrDs) was established in April 1999. The aim of this study is to describe the features of epidemiology, clinical manifestations and CSF biomarkers of human PrDs in Japan, including an additional period until September 2013.

Patients and Methods. We collected information on clinical, neuropathological, and molecular genetic data of patients suspected as having PrDs and analyzed by the Creutzfeldt-Jakob disease (CJD) Surveillance Committee, Japan, from April 1999 to September 2013.

Results. We have obtained the information of 4,281 patients. A total of 2,162 cases (50.5%) of PrDs was identified, including 1,655 cases of sporadic CJD (sCJD) (76.5%), 325 cases of genetic CJD (15.0%), 85 cases of GSS (3.9%), 4 cases of fatal familial insomnia (FFI) (0.2%), 84 cases of dura mater graft-associated CJD (dCJD) (3.9%), 1 case of variant CJD (vCJD) (0.04%), and 7 cases of unclassified CJD (0.3%). The overall annual incidence rate was 1.47 cases per million person-year. Genetic analysis was performed in 1,672 patients, and revealed that 402 cases had mutations in prion protein (PRNP) gene. The most frequent mutation was 187 cases of V180I mutation (46.5%), followed by 75 cases of P102L (18.7%), 58 cases of M232R (14.4%), 55 cases of E200K (13.7%), 7 cases of P105L, 5 cases of D178N, 2 cases of V203I, 1 case of R208H, 3 cases of V180I+M232R, 6 cases of insertion. Autopsy was performed 16.6% of total 1,616

patients who had died. Of total 147 dCJD cases in Japan, 84 cases appeared in this surveillance system. Only one case of vCJD with a history of a short stay in UK was identified in 2005. V180I is the most frequently occurring mutation in genetic PrDs in Japan, but has rarely been reported in other countries. P105L mutation and a valine-encoding polymorphic codon 129 in the *PRNP* have been reported only from Japan, and clinical features vary among patients.

Conclusion. Human PrDs in Japan were characterized by frequent occurrence of dCJD and relatively larger amount of patients with genetic PrDs which were composed of uncommon *PRNP* mutations in other countries.

References

1. Nozaki I, Hamaguchi T, Sanjo N, Noguchi-Shinohara M, Sakai K, Nakamura Y, Sato T, Kitamoto T, Mizusawa H, Moriwaka F, et al. Prospective 10-year surveillance of human prion diseases in Japan. *Brain* 2010; 133:3043-57; PMID:20855418; <http://dx.doi.org/10.1093/brain/awq216>
2. Higuma M, Sanjo N, Satoh K, Shiga Y, Sakai K, Nozaki I, Hamaguchi T, Nakamura Y, Kitamoto T, Shirabe S, et al. Relationships between clinicopathological features and cerebrospinal fluid biomarkers in Japanese patients with genetic prion diseases. *PLoS One* 2013; 8:e60003; PMID:23555862; <http://dx.doi.org/10.1371/journal.pone.0060003>
3. Hamaguchi, et al. *J Neurol Neurosurg Psychiatry*, In press.

P.185: Quinacrine and its bioavailability in treatment of prion disease

Martin Safarik,² Tibor Mosko,¹ Zbigniew Zawada,³ Eva Dvorakova,¹ Karel Holada,¹ and Jaroslav Sebestik²

¹First Faculty of Medicine; Charles University in Prague; Czech Republic; ²Institute of Organic Chemistry and Biochemistry; Academy of Sciences of the Czech Republic; Prague, Czech Republic; ³Institute of Chemical Technology; Prague, Czech Republic

Introduction. Quinacrine, drug based on 9-animoacridines, is capable to treat prion infection in dividing infected cells in vitro. However, it fails in attempts to treat prion infection in animals or humans. In our previous experiments we have shown that quinacrine can react with prion protein via cystein residues, which have potential to capture an aromatic acridine ring via their nucleophilic thiol groups.¹ In addition, quinacrine can interact with other compounds as well, e.g. glutathione in plasma or other compartments of human body. The reaction of glutathione with quinacrine has previously been observed. The thiol group of glutathione can serve as acridine moiety acceptor. We have previously characterized a product of interaction of quinacrine with glutathione (Glut-qui).¹ The level of glutathione in human plasma is approximately four orders of magnitude higher than in the standard in vitro conditions used for cell cultures. This suggests that glutathione can compete with the prion protein in vivo. In our study we have tested anti-prion capabilities of quinacrine and Glut-qui in in vitro infected cell cultures.

Material and Methods. CAD5 cells chronically infected with RML prions were treated with quinacrine and Glut-qui in wide range of concentrations. Cells were treated for 7 days in conditions ensuring space for dividing cells. Treated cells were

analyzed by the standard scrapie cell assay (SSCA) and western blot. The toxicity of quinacrine and Glut-qui on CAD5 cells was tested using cell counting kit-8 (Dojindo).

Results. Glut-qui had 45 times lower toxicity to CAD5 cells than quinacrine. Using SSCA we detected 28-fold decrease of its anti-prion activity. The anti-prion effect of the tested compounds was confirmed by western blot. The concentration of Glut-qui needed for complete disappearance of PrP^{Sc} from infected CAD5 culture was 20-fold higher compared to quinacrine.

Conclusion. The relative high concentrations of glutathione in plasma and body compartments can significantly decrease anti-prion capabilities of quinacrine and limits its bioavailability.

Acknowledgments. Funding: grant of the Czech science foundation GACR P303/12/1791 and project of Charles University in Prague PRVOUK-P24/LF1/3.

References

1. Zawada Z, Šafařík M, Dvořáková E, Janoušková O, Březinová A, Stibor I, Holada K, Bouř P, Hlaváček J, Sebestík J. Quinacrine reactivity with prion proteins and prion-derived peptides. *Amino Acids* 2013; 44:1279-92; PMID:23340659; <http://dx.doi.org/10.1007/s00726-013-1460-x>

P.186: Amplification of PrP^{Sc} from fatal familial insomnia brain tissue using wild-type human PrP^C brain substrate

Marcelo A Barria,¹ Alexander H Peden,¹ Richard S Knight,¹ James W Ironside,¹ Rona M Barron,² Jean C Manson,² and Mark W Head¹

¹National CJD Research & Surveillance Unit; Centre for Clinical Brain Sciences; School of Clinical Sciences; University of Edinburgh; UK; ²The Roslin Institute; University of Edinburgh; Easter Bush, UK

Keywords: prion disease, fatal familial insomnia, protein misfolding cyclic amplification

Human prion diseases are a group of neurodegenerative conditions classified as sporadic, familial or acquired, all of which are associated with misfolding and aggregation of the prion protein in the form of PrP^{Sc}. They comprise a wide range of clinical and neuropathological phenotypes including: Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträussler-Scheinker syndrome (GSS), fatal familial insomnia (FFI), among others. FFI is a rare inherited form closely associated with the D178N mutation in the prion protein gene (*PRNP*) linked with methionine (M) at position 129. Levels of detectable protease resistant prion protein (PrP^{res}) are known to be very low in the brain in the majority of FFI cases.

The search of molecular biomarkers for the early detection of the human prion disease has led to the development of cell-free conversion systems as rapid diagnostic tools. A recent study reported the detection of PrP^{Sc} in the CSF of several FFI cases by real-time quaking-induced conversion or RT-QUIC,¹ suggesting that this method could provide a useful clinical diagnostic tool for genetic as well as sporadic human prion diseases. However,

the biochemical properties of the RT-QUIC amyloid products have not been fully characterized.

Using an alternative in vitro conversion system, termed protein misfolding cyclic amplification or PMCA, we evaluated the amplification potential and biochemical properties (both pre- and post-amplification) of PrP^{Sc} from two FFI cases that had only very low levels of PrP^{res}. A single round of PMCA using a wild-type human PrP (*PRNP* 129 MM) substrate resulted in amplification, as judged by increased detection of PrP^{res} by Western blot analysis. Furthermore, the FFI PMCA reactions products retained the ability to seed a second round of PMCA. Moreover, the characteristic FFI PrP^{res} type was conserved during the amplification process. However, the protease resistance of the PMCA product was greater than that of the PrP^{Sc} in the sample that was used to seed the reaction. The significance of this increase in protease resistance is unknown, but we note that the in vitro amplification process involved the production of “wild-type PrP^{Sc}” rather than the D178N mutant PrP^{Sc} present in the seed.

References

1. Sano K, Satoh K, Atarashi R, Takashima H, Iwasaki Y, Yoshida M, Sanjo N, Murai H, Mizusawa H, Schmitz M, et al. Early detection of abnormal prion protein in genetic human prion diseases now possible using real-time QUIC assay. *PLoS One* 2013; 8:e54915; PMID:23372790; <http://dx.doi.org/10.1371/journal.pone.0054915>

P.187: Analysis of RNA expression profiles to identify molecular pathways underlying Creutzfeldt-Jakob disease pathogenesis

Anna Bartoletti Stella,^{1,2} Sabina Capellari,^{1,2} Patrizia Corrado,¹ Pascal F Durrenberger,³ Thomas Arzberger,⁴ Hans Kretzschmar,⁴ and Piero Parchi^{1,2}

¹DIBINEM, Dipartimento di Scienze Biomediche e Neuromotorie, Università di Bologna, Bologna, Italy; ²IRCCS, Istituto delle Scienze Neurologiche di Bologna; Bologna, Italy; ³Wolfson Neuroscience Laboratories, Imperial College London; London, UK; ⁴Zentrum für Neuropathologie und Prionforschung, Ludwig-Maximilians-Universität; Munich, Germany

Introduction. Functional genomics approaches applied to the study of RNA expression profile have revealed several abnormal molecular processes in prion diseases. Most published data, however, comes from studies on brain samples from prion-infected animals (mouse, cattle, and sheep),¹ whereas, to date, only one study has been carried out in humans.² To better characterize the transcriptional cascade associated with sporadic Creutzfeldt-Jakob disease (sCJD), the most common human prion disease, we studied the global gene expression profile in brain samples from sCJD affected patients. Differentially expressed genes (DEG) and altered pathways were then compared with previously published data to define common molecular mechanisms associated with animal and human prion diseases and, possibly, other neurodegenerative disorders.

Material and Methods. We obtained genome-wide RNA profiles from frontal cortex samples of 10 sCJD subjects and 10 controls with no neurological or psychiatric signs using Illumina

(Human Ref8 v2 Bead Chip) microarrays. After data validation by qPCR, we performed functional analysis of DEG, and annotation and ontology analyses using DAVID³ and GSEA⁴ tools. To compare our data with those previously reported, we analysed the lists of DEG with the same method.

Results. A comparison between sCJD and controls identified 344 DEG, 190 of which were up-regulated (Fold change>2) and 154 down-regulated (Fold change<0.5). 88 (25%) of these genes were also found altered in the only previous study on sCJD brains.² Interestingly, despite the relatively low percentage of shared deregulated gene between the two studies, functional analysis returned similar results. Most affected biological pathways in sCJD involved the down-regulation of genes associated with synaptic function, the overexpression of immune system pathways and the deregulation of transmembrane transport, homeostasis, carbohydrates and lipid metabolism. Moreover, a significant cluster of deregulated genes was linked to membrane trafficking involving Golgi and lysosome vesicle biogenesis and the trans-Golgi network. Noteworthy, the alteration of genes involved in immune system, lipid metabolism and endosomal-vacuolar pathway also characterized the transcriptional signature of prion-infected mice.

Conclusion. Our study confirms that major altered pathways in sCJD involve synaptic and immune system functions, but also suggests that an altered membrane trafficking features in the pathogenesis of prion disease. Interestingly, all these molecular pathways have also been associated with other neurodegenerative disorders such as Alzheimer's and Parkinson's disease.

References

1. Basu U, Guan LL, Moore SS. Functional genomics approach for identification of molecular processes underlying neurodegenerative disorders in prion diseases. *Curr Genomics* 2012; 13:369-78; PMID:23372423; <http://dx.doi.org/10.2174/138920212801619223>
2. Xiang W, Windl O, Westner IM, Neumann M, Zerr I, Lederer RM, Kretzschmar HA. Cerebral gene expression profiles in sporadic Creutzfeldt-Jakob disease. *Ann Neurol* 2005; 58:242-57; PMID:16049922; <http://dx.doi.org/10.1002/ana.20551>
3. Dennis G Jr., Sherman BT, Hosack DA, Yang J, Gao W, Lane HC, Lempicki RA. DAVID: Database for Annotation, Visualization, and Integrated Discovery. *Genome Biol* 2003; 4:3; PMID:12734009; <http://dx.doi.org/10.1186/gb-2003-4-5-p3>
4. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, Paulovich A, Pomeroy SL, Golub TR, Lander ES, et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proc Natl Acad Sci U S A* 2005; 102:15545-50; PMID:16199517; <http://dx.doi.org/10.1073/pnas.0506580102>

P.188: Co-localization of PrP, A-beta and tau in an autopsy case with features of both CJD and AD (a triple proteinopathy)

Beata Sikorska and Pawel P Liberski

Department of Molecular Pathology and Neuropathology; Medical University of Lodz; Lodz Poland

There are several molecular genetic and neuropathological links between Creutzfeldt Jakob disease and Alzheimer disease. Many various misfolded proteins were studied in both diseases but we decided to investigate the presence and co-localization of

misfolded proteins in an autopsy case with features of both CJD and AD. We used routine neuropathology, immunohistochemistry and confocal laser microscopy in a formalin fixed paraffin embedded human brain tissues.

Immunohistochemical reaction showed synaptic pattern of the PrP^{Sc} deposits in the cerebellar and the cerebral cortices and in the basal ganglia.

No plaque-like deposits or kuru plaques were observed apart from focal enhancement of PrP^{Sc} immunoreactivity in the areas of senile plaques. Immunohistochemistry revealed amyloid beta—immunoreactive plaques in the frontal, temporal, occipital and parietal cortex. Phosphorylated tau—reactive neurofibrillary tangles and neuropil threads were noted in the highest numbers in the transentorhinal cortex, hippocampal areas and occipito-temporal gyrus.

Confocal laser microscopy revealed the presence of PrP^{Sc} and amyloid beta in/around amyloid plaques in CJD-AD but without real co-localization. We observed co-localization of PrP^{Sc} and APP in dystrophic neurites inside the plaques as well as in neuropil. Partial co-localization of hyperphosphorylated MAP-tau, was present in dystrophic neurites but not in NFTs. Additionally we compared these results with amyloid plaques in AD and amyloid plaques in a subtype of CJD with kuru plaques.

Conclusion. The co-localisation of misfolded proteins in amyloid plaques is observed mainly in dystrophic neurites.

P.189: Transgenic mouse expressing a novel Gerstmann-Sträussler-Scheinker disease *Prnp* allele with a partial duplication of the PrP hydrophobic domain

Robert CC Mercer,^{1,2} Charles E Mays,¹ Nathalie Daude,¹ Hristina Gapeshtina,¹ Serene L Wohlgemuth,¹ Jing Yang,¹ Neil R Cashman,³ Michael B Coulthart,⁴ and David Westaway^{1,2,5,6}

¹Centre for Prions and Protein Folding Diseases; University of Alberta, Edmonton, AB Canada; ²Department of Medicine (Neurology), University of Alberta; AB Edmonton, Canada; ³Division of Neurology, University of British Columbia; Vancouver, BC Canada; ⁴Canadian Creutzfeldt-Jakob Disease Surveillance System; Public Health Agency of Canada; Winnipeg, MB Canada; ⁵Centre for Neuroscience; University of Alberta; Edmonton, AB Canada; ⁶Department of Biochemistry, University of Alberta; Edmonton, AB Canada

Background. Gerstmann-Sträussler-Scheinker (GSS) disease is a rare, genetic prion disease that often manifests as progressive cerebellar ataxia and eventual cognitive decline. GSS can be distinguished from other prion diseases by the presence of widespread multicentric amyloid plaques containing prion protein fragments. Recently, a novel *PRNP* mutation was identified in a 34-year-old male.¹ This individual, who is homozygous for valine at residue 129, contains an 8 amino acid duplication of residues 122-129 (inserted between 129/130) extending the length of the hydrophobic domain (HD), the most highly conserved region of PrP. This expansion mutation is believed to have occurred

de novo. We have created transgenic mice expressing the murine version of this allele and present biochemical and histopathological analysis of these animals as well as findings from cell culture models.

Work plan. Using independent lines of Tg mice expressing the mutant allele in the context of a valine codon at position 128 (human codon 129) we determined the age of onset of neurodegeneration and the transmissibility of the disease by intracranial inoculation. Histopathological analyses included immunostaining for PrP^{Sc}, assessment of vacuolation by H&E staining, determining the presence/extent of amyloid formation with Congo red and thioflavin S and immunostaining for glial fibrillary acidic protein (GFAP) to determine the extent of gliosis. We profiled PrP by immunoblot following exposure to PNGase F and proteinase K, and also assessed alternative membrane topologies of PrP (^{C_{tm}}PrP) arising from expansion of the hydrophobic domain. The susceptibility of cell culture models to stress-induction was analyzed for both mutant and control alleles.

Results. Two independent Tg lines carrying the partial duplication of the HD ("Tg-Dupl" mice) developed a spontaneous neurologic syndrome at ages >300 days. This was associated with the accumulation of protease-resistant PrP fragments. Control Tg lines expressing mouse PrP with valine at position 128 did not develop disease.

Conclusion. "Tg-Dupl" mice may comprise a new model for a variant form of human GSS.

References

1. Hinnell C, Coulthart MB, Jansen GH, Cashman NR, Lauzon J, Clark A, Costello F, White C, Midha R, Wiebe S, et al. Gerstmann-Straussler-Scheinker disease due to a novel prion protein gene mutation. *Neurology* 2011; 76:485-7; PMID:21282596; <http://dx.doi.org/10.1212/WNL.0b013e31820a0ab2>

P.190: Occupational exposures to prion diseases in healthcare and laboratory workers

Jennifer Thorpe,¹ Katy Sinka,¹ Robert Will,²
Anna Molesworth,² and Jan Mackenzie²

¹Public Health England; London, UK; ²National CJD Research and Surveillance Unit, Edinburgh, UK

Background. Epidemiological studies have not found an association between occupational exposure and the risk of prion disease, however the possibility of this occurring in healthcare and laboratory settings remains an important concern. Public Health England (PHE), in collaboration with the National CJD Research and Surveillance Unit (NCJDRSU) have created a registry to improve the national evidence base regarding exposure to CJD and other prion diseases.

Methods. The registry has two parts, including the prospective long term monitoring of healthcare and laboratory workers who are known to have incurred occupational exposures and secondly, the retrospective review of possible occupational exposures of CJD cases who have been healthcare or laboratory workers.

The prospective element of the registry involves voluntary reporting of occupational exposures as they occur. In the retrospective element, for CJD patients who have been healthcare workers, overlaps between their employment and all hospital procedures and inpatient stays of other CJD patients are identified by NCJDRSU. These time and place overlaps indicate 'potential exposure periods'. If any such periods are identified, a further investigation with the hospital can be undertaken to identify any possible occupational links between the CJD infected healthcare worker and the concurrent CJD patient. For CJD patients who have been laboratory workers, investigations are conducted with the relevant laboratory to identify any possible exposures and accidental injuries involving transmissible spongiform encephalopathy (TSE) infected animals or tissues during their employment.

Results. To date there have been three voluntary reports to the prospective register from different health and laboratory occupations. The retrospective review has identified a number of potential exposure periods between healthcare workers who developed CJD and other CJD patients. PHE in collaboration with the NCJDRSU will shortly be contacting the relevant occupational health departments to investigate if these healthcare workers reported any relevant occupational exposures and will present the findings. Following a review of four laboratory workers, the lookback has been completed and did not find any reported occupational health exposures.

Conclusion. CJD and prion diseases are rare diseases with a long incubation periods, and many more years of surveillance and investigation are needed to understand occupational exposures as a transmission risk. This study describes the approach taken in the UK.

P.191: Prion protein genetic variation in 50,000 humans

Eric Minikel,^{1,2,3} Sonia Vallabh,^{1,4} Exome Aggregation Consortium (ExAC), Monkol Lek,^{2,3} and Daniel G MacArthur^{2,3}

¹Prion Alliance; Cambridge, MA USA; ²Broad Institute; Cambridge, MA USA;

³Analytical and Translational Genetics Unit, Massachusetts General Hospital; Boston, MA USA; ⁴Center for Human Genetic Research, Massachusetts General Hospital; Boston, MA USA

Keywords: genetics, PrP

Here we provide the first comprehensive survey of genetic variation in PRNP in a large control dataset. We have aggregated exome sequence data from ~50,000 individuals from ~60 different sequencing studies and have performed joint variant calling to identify rare sequence variants. The dataset includes controls as well as cases ascertained for a variety of phenotypes, however, none of these phenotypes are neurodegenerative in nature, and thus we expect that this dataset likely represents a neutral view of prion protein genetic variation in the population, complementary to data ascertained from individuals with prion disease.

Given the reported incidence of prion disease in the population (~1e-6 per year), the proportion of reported cases associated with genetic mutations (~15%) and the life expectancy of mutation carriers (~50 years), the expected number of alleles with known pathogenic mutations to be observed by chance in a dataset of this size is approximately 0.5. When we consider the most frequently reported prion disease mutations (e.g. P102L, D178N, E200K), we observe 0 alleles, consistent with full penetrance. However we do observe alleles of several other reported disease mutations (V180I, T188R, V203I, R208H, V210I, Q212P), suggesting that some of these mutations may not be fully penetrant, and/or that there exists some degree of clinical under-ascertainment of genetic prion disease cases. We also observe tens of novel missense mutations including several at known or suspected pathogenic codons but with different amino acid substitutions (e.g. R148C, E196A, E200D, and P238A), which may support the hypothesis that pathogenicity is associated with increased charge or hydrophobicity. Finally, we observe two novel truncating mutations which are currently undergoing validation.

P.192: A Korean patient with sporadic Creutzfeldt-Jakob disease confirmed using real-time quaking-induced conversion assay

Jae Woo Kim,¹ Dong-Hyun Shim,¹ Sang-Myung Cheon,¹ and Yong-Sun Kim²

¹Department of Neurology, Dong-A University Hospital; Busan, Korea; ²Ilson Institute of Life Science, College of Medicine, Hallym University; Chuncheon, Gangwon, Korea

Introduction. The definite diagnosis of Creutzfeldt-Jakob disease (CJD) requires pathological confirmation. Real-time quaking-induced conversion (RT-QUIC) is recently developed technology to detect the abnormal form of prion protein in CSF from sporadic CJD patients. We report a patient with sporadic CJD who was diagnosed using RT-QUIC.

Case. A 66-year-old man was admitted due to dizzy sense and gait disturbance for 1 month. He complained of dizziness of non-whirling type and ataxia when he walked since 1 month before admission. He also showed repetitive saying and purposeless behavior which were progressed. On examination slurred speech and frontal lobe releasing signs were noted. He showed a wide based and ataxic gait more on the left side. He also complained of distorted vision. Mini-mental state examination score was 17/30. Brain MRI revealed high signal intensities in multiple cortical area and both basal ganglia. Electroencephalography showed triphasic waves. 14-3-3 protein was positive in CSF. The level of Abeta 42 was 555.7 pg/ml and Tau 16732 pg/ml. Abnormal prion protein in CSF was elevated which was measured using RT-QUIC 4 times consecutively. Mutation was not found in PRNP gene. The genotype at 129 codon was Met/Met.

Conclusion. The RT-QUIC is a useful method for detection of sporadic CJD patient as an early, rapid and specific assay

P.193: Interlaboratory optimisation, harmonisation and standardisation of CSF RT-QuIC in the diagnosis of sCJD

Lynne I McGuire,¹ Song Shi,² Ilaria Poggiolini,³ Michele Equestre,⁴ Silvia Suardi,⁵ Katarina Grznarova,⁶ Anna Ladogana,⁴ Stéphane Haïk,⁶ Piero Parchi,³ Maurizio Pocchiari,⁴ Fabrizio Tagliavini,⁵ Jean-Philippe Brandel,⁶ Robert Will,¹ Richard S Knight,¹ and Alison JE Green¹

¹The National CJD Research & Surveillance Unit, University of Edinburgh; Edinburgh, Scotland, UK; ²Centre for Neuropathology and Prion Research, Ludwig-Maximilians-Universität; Munich, Germany; ³Department of Biomedical Science and Neuromotor Sciences, University of Bologna; Bologna, Italy; ⁴Department of Neurological Sciences, Istituto Superiore di Sanità; Rome, Italy; ⁵National Neurological Institute "Carlo Besta" (INCB); Milan, Italy; ⁶Laboratory investigations of Alzheimer's disease and Prion diseases, Groupe Hospitalier Pitié-Salpêtrière; Paris, France

Real-time quaking-induced conversion (RT-QuIC) is a technique which exploits the ability of PrP^{Sc} in brain tissue or CSF to induce recombinant PrP to change shape and aggregate over time monitored by the binding of Thioflavin T (ThT). Studies have shown that cerebrospinal fluid (CSF) samples from hamsters inoculated with experimental scrapie and from patients with sporadic Creutzfeldt-Jakob disease (sCJD) can be correctly identified using RT-QuIC.^{1,2,3}

At the National CJD Research & Surveillance unit (NCJDRSU) we have completed a retrospective study and a prospective audit investigating the value of RT-QuIC in the diagnosis of sCJD.³ These two independent studies have shown that analysing CSF samples from patients with suspected sCJD using this technique has enabled those patients with disease to be identified with a high degree of sensitivity and specificity. This is a major advance in the development of a disease-specific pre-mortem diagnostic test for sCJD and there is considerable interest in establishing this test as a routine diagnostic test in Europe. Funding from JPND research initiative has allowed us alongside collaborators from across Europe, to undertake a study to look at the optimisation, harmonisation and standardisation of the RT-QuIC technique in the diagnosis of sCJD. It is important that RT-QuIC is optimised in terms of analytical performance and it is crucial to ensure that there is a consistency in results between laboratories. As part of this study, six laboratories across Europe (UK, Germany, France and 3 centres in Italy) have taken part in a blinded trial study. Once each laboratory had established the technique they were sent 10 blinded CSF samples to test. Using standardised criteria the samples were classified as either positive or negative for sCJD. These results were very encouraging suggesting that the RT-QuIC technique is both robust and highly transferrable between laboratories. Additional blinded trial studies are planned and will increase and widen our data set including results from further European centres and collaborating International centres which have established the RT-QuIC technique. Our blinded trial results and some of our steps

towards optimisation, harmonisation and standardisation of the RT-QuIC technique in the diagnosis of sCJD will be discussed.

References

1. Atarashi R, Sano K, Satoh K, Nishida N. Real-time quaking-induced conversion: a highly sensitive assay for prion detection. *Prion* 2011; 5:150-3; PMID: 21778820; <http://dx.doi.org/10.4161/pri.5.3.16893>
2. Wilham JM, Orrú CD, Bessen RA, Atarashi R, Sano K, Race B, Meade-White KD, Taubner LM, Timmes A, Caughey B. Rapid end-point quantitation of prion seeding activity with sensitivity comparable to bioassays. *PLoS* 2010; 6:e1001217; PMID: 21152012; <http://dx.doi.org/10.1371/journal.ppat.1001217>
3. McGuire LI, Peden AH, Orrú CD, Wilham JM, Appleford NE, Mallinson G, Andrews M, Head MW, Caughey B, Will RG, et al. Real time quaking-induced conversion analysis of cerebrospinal fluid in sporadic Creutzfeldt-Jakob disease. *Ann Neurol* 2012; 72:278-85; PMID:22926858; <http://dx.doi.org/10.1002/ana.23589>

P.194: Computer-aided design of ligands targeting RNA polyCAG expansion tracts in Huntington's disease

Anna Bochicchio,¹ Giulia Rossetti,^{1,2,3,4} Francesco Musiani,^{1,5} Paolo Carloni,¹ Sergio Pantano,⁶ Sybille Krauss,⁷ and N Jon Shah⁸

¹Computational Biophysics, German Research School for Simulation Sciences, Jülich, Germany; Institute for Advanced Simulation IAS-5, Computational Biomedicine, Forschungszentrum Jülich; Jülich, Germany; ²Institute for Research in Biomedicine (IRB Barcelona); Barcelona, Spain; ³Joint IRB-BSC Program in Computational Biology; Barcelona, Spain; ⁴Jülich Supercomputing Centre (JSC), Forschungszentrum Jülich; Jülich, Germany; ⁵Laboratory of Bioinorganic Chemistry, Department of Pharmacy and Biotechnology (FaBIT), University of Bologna; Bologna, Italy; ⁶Institut Pasteur de Montevideo; Montevideo, Uruguay; ⁷German Center for Neurodegenerative Diseases (DZNE); Bonn, Germany; ⁸Institute of Neuroscience and Medicine-4, Forschungszentrum Jülich; Jülich, Germany

Huntington's disease (HD) is an autosomal neurodegenerative genetic disorder characterized by motor disturbance, cognitive loss, and psychiatric manifestations and affects 4-10 in 100,000 individuals in European populations.

The gene responsible for the disease (HTT) encodes the Huntingtin protein (Htt), essential for brain development. The disease is caused by an expanded CAG repeat in the 5'-end of HTT, which encodes an abnormally long polyglutamine (polyQ) tract in the Htt protein. HD penetrance is correlated to the number *n* of CAG repeats. Mounting evidence is emerging that HTT mRNA transcripts with *n*>36 (hereafter RNA_{CAG}) (i) contribute to the pathogenesis of CAG disorders; (ii) aberrantly regulate several cellular mechanisms; (iii) bind to proteins in repeat size-dependent manner to form pathological complexes. As recently shown by S.Krauss's laboratory, one of those involves the Midline-1 protein (MID1), in complex with protein phosphatase 2A (PP2A)¹, which leads to an overproduction of aberrant Htt protein¹. This effect can be significantly reduced by knock-down of MID1.¹ Hence, the design of novel ligands with high affinity for RNA_{CAG}, interfering with RNA_{CAG}/protein pathological complexes, constitutes a promising drug target strategy against HD.

The aim of this project is to identify high affinity ligands targeting specifically RNA_{CAG} by using computational methods.

We will present preliminary results regarding docking studies on ligands/RNA_{CAG} complexes and development of Coarse Grained models to characterize the flexibility of RNA_{CAG} below and above the disease threshold.

The selected ligands will be tested in vitro and in HD transgenic mouse models in vivo. MRI experiments on healthy and transgenic mice brains in the presence and absence of the identified ligands will also be performed.

References

1. Krauss S, Griesche N, Jastrzebska E, Chen C, Rutschow D, Achmüller C, Dorn S, Boesch SM, Lalowski M, Wanker E, et al. Translation of HTT mRNA with expanded CAG repeats is regulated by the MID1-PP2A protein complex. *Nat Commun* 2013; 4:1511; PMID:23443539; <http://dx.doi.org/10.1038/ncomms2514>

P.195: Different patterns of microglial cells activation in sporadic Creutzfeldt-Jakob disease subtypes

Giulia Pasini,¹ Sergio Ferrari,¹ Marco Chilosi,² Matilde Bongianni,¹ Salvatore Monaco,¹ and Gianluigi Zanusso¹

¹Department of Neurological and Movement Sciences; University of Verona; Verona, Italy; ²Department of Pathology and Diagnostics; University of Verona; Verona, Italy

Introduction. Microglial activation has been described in sporadic and variant Creutzfeldt-Jakob disease (CJD) and related to disease-specific forms of the prion protein (PrP^{Sc}). These PrP^{Sc} forms act as prion strains showing specific tropisms towards neural or lymphatic tissue. In the present study, we characterized microglia phenotypes in definite cases of sCJD using specific markers and we investigated whether distinct sCJD subtypes might be distinguished based on microglial phenotype.

Methods. We carried out a neuropathologic and biochemical study on 16 patients with sporadic CJD, including all possible genotypes at codon 129 of the prion protein gene. We obtained from each subject formalin fixed sections from frontal and occipital cortices, cerebellum, thalamus and basal ganglia. Serial sections were immunostained with monoclonal antibodies directed to PrP, GFAP and to different monocyte/macrophage markers such as CD68, CD11c, CD163. Quantitative analysis of microglial expression was also determined by Western blot analysis.

Results. In sCJD cases, MM and MV at codon 129 with a PrP^{Sc} type 1, immunohistochemistry showed a severe degree of microglia activation while in sCJD with a PrP^{Sc} type 2 was moderate. In particular, in sCJD with a PrP^{Sc} type 1 microglial cells were characterized by rounded cell bodies, shorted and dilated cell processes. In contrast, in sCJD cases with PrP^{Sc} type 2, microglia was mainly ramified and formed small clusters of activated cells in subcortical white matter and in perivascular spaces. The highest extent of microglial proliferation and activation was observed in cases with status spongiosus, in contrast in cortical

areas with a coarse pattern of PrP deposition only rare microglial cells were observed.

Conclusion. These findings support the view that the phenotypic heterogeneity of microglia in different subtypes of sporadic CJD is influenced by PrP^{Sc} conformers. Moreover, the variable extent of microglia activation in distinct sCJD subtypes indicates specific prion strain tropisms and might provide clues on their potential properties of spreading prion infectivity.

P.196: Comparison of brushes and swabs for olfactory mucosa sampling and RT-QuIC analysis of patients with Creutzfeldt-Jakob disease

Matilde Bongianni,^{1,2,†} Christina D Orrù,^{1,3,†} Giovanni Tonoli,⁴ Andrew G Hughson,¹ Sergio Ferrari,² Bradley R Groveman,¹ Luca Sacchetto,⁴ Michele Fiorini,² Maurizio Pocchiari,⁵ Salvatore Monaco,² Byron Caughey,¹ and Gianluigi Zanusso²

¹Laboratory of Persistent Viral Diseases, Rocky Mountain Laboratories, National Institute for Allergy and Infectious Diseases, National Institutes of Health; Hamilton, Montana, USA; ²Department of Neurological and Movement Sciences, University of Verona; Verona, Italy; ³Department of Biomedical Sciences, University of Cagliari; Cagliari, Italy; ⁴Clinica Otorinolaringoiatrica, Azienda Ospedaliera Universitaria Integrata; Verona, Italy; ⁵Department of Cell Biology and Neurosciences, Istituto Superiore di Sanità; Rome, Italy

[†]These authors contributed equally to this study

Introduction. Definitive diagnosis of Creutzfeldt-Jakob disease (CJD) in living patients remains a challenge. In a recent study, we identified prion seeding activity in olfactory mucosa (OM) of CJD patients by nasal brushing coupled with Real Time Quaking induced Conversion (RT-QuIC), obtaining 100% specificity and ≥97% sensitivity for the detection of CJD. OM samples were collected with a sterile disposable Cyto-brush (Kito-Brush, Kaltek) that might cause mild discomfort for the patients. Therefore, we used less abrasive short nylon fiber Flocked swabs (Flocked swab, Copan Diagnostics) for sampling.

Materials and methods. We collected OM samples in 17 CJD patients. To ensure efficient OM sample collection, each patient underwent samplings of OM with Flocked swabs followed by Cyto-brushes. OM samples were processed and analyzed by RT-QuIC.

Results. Using Cyto-brushes 16 out of 17 CJD cases were positive by RT-QuIC analysis, while Flocked swabs were positive for 15 out of 17 CJD samples. In one patient, OM samples were negative for both Cyto-brush and Flocked swab samplings. Neither OM sampling technique produced false positives.

Conclusion. Overall, Cyto-brushes and Flocked swabs gave comparable sensitivity. However, for one patient the Cyto-brush was positive by RT-QuIC but the two consecutive Flocked swabs were negative. Although the efficacy of OM sampling with Flocked swabs needs further evaluation, this collection method shows promise as a gentle, patient friendly alternative to sampling by Cyto-brush. Our current recommendation is to adopt

a combination of Flocked swabs and Cyto-brushes to maximize the collection of OM material and minimize patient discomfort.

Acknowledgments. The authors acknowledge Copan Diagnostics for their collaboration.

P.197: Role of desolvation and hydrophobicity in early stages of formation of toxic Aβ-oligomers

Nikolay Blinov,^{1,2} Neil R Cashman,³ and Andriy Kovalenko^{1,2}

¹National Institute for Nanotechnology; Edmonton, AB Canada; ²Department of Mechanical Engineering, University of Alberta; Edmonton, AB Canada; ³Department of Medicine, University of British Columbia; Vancouver, BC Canada

Aβ oligomers are considered an important therapeutic target against Alzheimer's disease.¹ Elucidation of 3D-structure of the oligomers can provide a basis for inhibition of their toxicity and interference with oligomerization pathways. Experimental characterization of the oligomers is impeded by their possible transient character and structural flexibility. Molecular modeling provides a valuable insight into oligomerization mechanisms and structural characteristics of oligomers and amyloid fibrils.² General principles governing formation of oligomers have been deduced from molecular studies of fibril-forming fragments of Aβ peptides. These studies highlight the role of hydrophobic and electrostatic effects in the initial stages of oligomerization.²

To extend our understanding of oligomerization to the full length Aβ peptides in physiological conditions, we use a novel approach providing prediction of the propensity for aggregation of amyloidogenic peptides, based on the 3D-RISM-KH molecular theory of solvation which efficiently and accurately describes solvation effects with consistent account for solvent molecular specificities, composition, and thermodynamic state.³ We validated the theory on thermodynamics and solvation structure of β-sheet oligomers, Aβ and prion amyloid fibrils, and on prediction of binding modes of antiprion compounds.⁴⁻⁷ Here, we use this methodology to build/screen plausible models of Aβ (toxic) oligomers with account for restraints derived from conformational antibody-oligomer binding experiments, as well to study kinetics and thermodynamics of initial stages of oligomerization.

We also show that the balance between hydrophobicity and electrostatic interactions (including the interactions between salt bridge forming residues) for full length Aβ peptides can differ from that for short fragments. In particular, initial deposition of Aβ peptides on preformed oligomers can be driven by electrostatic rather than hydrophobic effects. Implications of these new findings on structural characteristics of Aβ oligomers are discussed.

References

1. Haass C, Selkoe DJ. Soluble protein oligomers in neurodegeneration: lessons from the Alzheimer's amyloid beta-peptide. *Nat Rev Mol Cell Biol* 2007; 8:101-12; PMID:17245412; <http://dx.doi.org/10.1038/nrm2101>
2. Straub JE, Thirumalai D. Principles governing oligomer formation in amyloidogenic peptides. *Curr Opin Struct Biol* 2010; 20:187-95; PMID:20106655; <http://dx.doi.org/10.1016/j.sbi.2009.12.017>

3. Kovalenko, in: *Molecular Theory of Solvation*, Hirata (ed.), Kluwer, Dordrecht 2003; 169-275.
4. Blinov N, Dorosh L, Wishart D, Kovalenko A. Association thermodynamics and conformational stability of beta-sheet amyloid beta(17-42) oligomers: effects of E22Q (Dutch) mutation and charge neutralization. *Biophys J* 2010; 98:282-96; PMID:20338850; <http://dx.doi.org/10.1016/j.bpj.2009.09.062>
5. Blinov, et al. *Mol Simul* 2011; 37:718-28; <http://dx.doi.org/10.1080/08927022.2010.544306>
6. Yamazaki T, Blinov N, Wishart D, Kovalenko A. Hydration effects on the HET-s prion and amyloid-beta fibrillous aggregates, studied with three-dimensional molecular theory of solvation. *Biophys J* 2009; 95:4540-8; PMID: 18689456; <http://dx.doi.org/10.1529/biophysj.107.123000>
7. Nikolić D, Blinov N, Wishart D, Kovalenko A. 3D-RISM-Dock: A New Fragment-Based Drug Design Protocol. *J Chem Theory Comput* 2012; 8:3356-72; <http://dx.doi.org/10.1021/ct300257v>

P.198: Immunohistochemical and neuropathological analyses of the prion protein in genetic Creutzfeldt-Jakob disease with V180I mutation

Masaki Hizume,¹ Nobuo Sanjo,¹ Katsuya Satoh,² Tsuyoshi Hamaguchi,³ Masahito Yamada,³ Yasushi Iwasaki,⁴ Atsushi Kobayashi,⁵ Tetsuyuki Kitamoto,⁵ and Hidehiro Mizusawa¹

¹Tokyo Medical and Dental University Graduate School of Medical and Dental Sciences; Tokyo, Japan; ²Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan; ³Kanazawa University Graduate School of Medical Science; Kanazawa, Japan; ⁴Aichi Medical University; Nagakute, Japan; ⁵Tohoku University Graduate School of Medicine; Sendai, Japan

Keywords: genetic Creutzfeldt-Jakob disease, V180I mutation, fPrP 11-12, MM2C

Introduction. Prion protein (PrP) deposition is considered a neuropathological confirmation of prion disease. Genetic Creutzfeldt-Jakob disease (gCJD) with V180I mutation is the most frequently occurring mutation in Japan, and the presence of PrP in the brain or cerebrospinal fluid of individuals with this condition is reportedly low. In this study, we analyzed the brains of patients with V180I mutations, post-mortem, by using Western blot and immunohistochemistry.

Material and Methods. Brain sections from 8 patients with V180I and brain homogenates from 7 patients with V180I were analyzed and compared with those from patients with MM1 sporadic CJD (sCJD), MM2-cortical sCJD, and P102L, D178I, E200K, and M232R mutations. The proteinase K-treated brain homogenates were transferred to a membrane after SDS-PAGE and probed with #71 original antibody (epitope: codon 215-220) and 3F4 antibody (epitope: codon 106-110). Brain sections were evaluated based on the degree of spongiform changes and vacuolar diameter, observed after hematoxylin and eosin staining, and the amount of PrP deposition was detected by immunohistochemistry.

Results. The immunoreactivity of brain homogenates from patients with V180I in Western blotting was very weak compared to those from patients with MM1 and MM2C. Diglycosylated PrP^{Sc} was not detected and the migration distance of unglycosylated PrP^{Sc} was slightly shorter than that of type 2 PrP^{Sc}. In one

case, the migration distance was equal to that of type 2 PrP^{Sc}. We detected carboxyl-terminal fragments (fPrP 11-12) probed with #71 antibodies, and the ratio of fPrP 11-12 to full-length PrP in V180I samples (84%) was higher than those in the other prion disease samples (0-11%). The degree of spongiform change varied between sulci, and synaptic PrP deposition was detected more frequently in the hippocampus (75%) than in the cortices. In the V180I samples in which the unglycosylated and type 2 PrP^{Sc} bands had the same migration distance, large confluent vacuoles and perivacuolar PrP deposits were detected, which was similar to those in cases with MM2C-sCJD. The immunohistological sensitivity to PrP was higher with 3F4 antibody than with #71 antibody.

Conclusion. The observed pathological features of V180I were: (1) lack of diglycosylated PrP^{Sc} in Western blotting, (2) greater migration distance of unglycosylated PrP^{Sc} in SDS-PAGE, which was slightly shorter than that of type 2 PrP^{Sc}, (3) prominent ratio of fPrP 11-12 to full length PrP, (4) PrP deposition predominantly in the hippocampus, and (5) possible coexistence with MM2C-like PrP^{Sc} in some cases.

P.199: Determination of the sample-storage dependent stability on the RT-QuIC signal

Maria Cramm,¹ Matthias Schmitz,¹ Bjoern Schröder,² Alex Raeber,² Franziska Kuhn,² and Inga Zerr¹

¹Department of Neurology, Clinical Dementia Center and DZNE Georg-August University Göttingen; Göttingen, Germany; ²Prionics AG; Zurich, Switzerland

Keywords: Creutzfeldt-Jakob disease, cerebrospinal fluid, real-time quaking-induced conversion, prion protein

Background. Creutzfeldt-Jakob disease (CJD) is caused by scrapie prion protein (PrP^{Sc}), the pathogenic form of cellular prion protein (PrP^C), which is able to convert PrP^C into PrP^{Sc}.¹ About 85% of the CJD cases appear sporadic (sCJD).² Real-time quaking-induced conversion (RT-QuIC) uses the self-propagating properties of PrP^{Sc} to amplify it up to a detectable limit, by the use of recombinant prion protein (rPrP) as a substrate and intermittent automated shaking. Fluorescence detection of aggregated PrP^{Sc} monitors amplification in real-time.³ Recent studies showed the potential of RT-QuIC to detect PrP^{Sc} in CSF of CJD patients.^{4, 5, 6, 7} In the present study we analyzed the influence of sample storage conditions (StC) on the stability of the RT-QuIC signal.

Materials and Methods. Sporadic CJD patients were classified as definite cases by neuropathological examination. Control patients were proven to have an alternative diagnosis. Groups including short and long-term stored CSF samples were created (N=12 sCJD samples per StC). CSF sample was supplemented with working buffer including rPrP and incubated in a plate reader with intermittent shaking cycles. Aggregate formation was determined by measuring the fluorescence signal in relative fluorescence units (rfu). In a preceded study we estimated the best

cut-off value to be 10,000 rfu and also applied that cut-off here, to define a CSF sample as sCJD-positive.⁷

Results and Conclusion. First the number of positive samples (as defined by cut-off) was determined per StC and compared, using analysis of variance. No significant influence on the number of positive samples was observed for all StCs. Following the RT-QuIC signals of all positive sCJD samples were determined per StC. No significant influence on the RT-QuIC signal was observed for all of them. From our data we conclude that the RT-QuIC signal is stable in the face of different CSF sample storage conditions.

References

1. Prusiner SB. Prions. *Proc Natl Acad Sci U S A* 1998; 95:13363-83; PMID:9811807; <http://dx.doi.org/10.1073/pnas.95.23.13363>
2. Parchi P, Strammiello R, Notari S, Giese A, Langeveld JP, Ladogana A, Zerr I, Roncaroli F, Cras P, Ghetti B, et al. Incidence and spectrum of sporadic Creutzfeldt-Jakob disease variants with mixed phenotype and co-occurrence of PrP^{Sc} types: an updated classification. *Acta Neuropathol* 2009; 118:659-71; PMID:19718500; <http://dx.doi.org/10.1007/s00401-009-0585-1>
3. Atarashi R, Sano K, Satoh K, Nishida N. Real-time quaking-induced conversion: a highly sensitive assay for prion detection. *Prion* 2011; 5:150-3; PMID:21778820; <http://dx.doi.org/10.4161/pri.5.3.16893>
4. Atarashi R, Satoh K, Sano K, Fuse T, Yamaguchi N, Ishibashi D, Matsubara T, Nakagaki T, Yamanaka H, Shirabe S, et al. Ultrasensitive human prion detection in cerebrospinal fluid by real-time quaking-induced conversion. *Nat Med* 2011; 17:175-8; PMID:21278748; <http://dx.doi.org/10.1038/nm.2294>
5. McGuire LI, Peden AH, Orrú CD, Wilham JM, Appleford NE, Mallinson G, Andrews M, Head MW, Caughey B, Will RG, et al. Real time quaking-induced conversion analysis of cerebrospinal fluid in sporadic Creutzfeldt-Jakob disease. *Ann Neurol* 2012; 2:278-85; PMID: 22926858; <http://dx.doi.org/10.1002/ana.23589>
6. Sano K, Satoh K, Atarashi R, Takashima H, Iwasaki Y, Yoshida M, Sanjo N, Murai H, Mizusawa H, Schmitz M, et al. Early detection of abnormal prion protein in genetic human prion diseases now possible using real-time QUIC assay. *PLoS One* 2013; 8:e54915; PMID:23372790; <http://dx.doi.org/10.1371/journal.pone.0054915>
7. Cramm, et al. Poster No.: HD.20. Prion 2013

P.200: Clinical expression of BSE in mouse models is unrelated to hallmarks of prion diseases

Christelle Jas-Duval,^{1,2} Jacqueline Mikol,¹

Sophie Luccantoni-Freire,¹ Christine Defer,²

Jean-Jacques Huart,² Paul Brown,¹ Jean-Philippe Deslys,¹
and Emmanuel E Comoy¹

¹CEA, Institute of Emerging Diseases and Innovative Therapies (iMETI), Division of Prions and Related Diseases (SEPIA); Fontenay-aux-Roses, France; ²EFS-Nord de France; Lille, France

Background. In 1997, we demonstrated that intracerebral inoculation of cattle BSE to C57Bl/6 mice induced neurological disease, even though half of the recipient animals were devoid of PrPres (Lasmez et al., 1997). PrPres appeared with shortening incubation periods after subsequent passages, suggesting that detectable PrPres should be considered as an indicator of strain virulence rather than as a specific (and required) marker of prion diseases. Subsequent blood risk studies showed that cynomolgus macaques exposed to vCJD-contaminated blood products developed either classical vCJD or a novel neurological disease without detectable PrPres. In our continuing studies of this phenomenon, we now report the results of intravenous inoculations of several

different wild-type mouse strains with bovine or primate BSE source material.

Materials and Methods. Brains from one bovine and one cynomolgus monkey showing clinical signs of BSE were sonicated and ultracentrifuged at 188,000 g for 1 hour. Resulting pellets and supernatants (equivalent to 2 mg of brain / mouse) were intravenously injected to PrP^{+/+} Swiss, PrP^{+/-} and PrP^{+/+} C57Bl/6N mice, totalling 12 separate bioassays. PrPres was detected using conventional ELISA, western blotting and IHC procedures. Pathology was studied on formalin-fixed brain tissues.

Results. The transmission rate of BSE in those 12 experiments ranged from 0% (PrP^{+/-} C57Bl/6N—cattle BSE—supernatant or pellet: no clinical sign, no spongiosis and no PrPres) to 100% (Swiss—primate BSE—supernatant or pellet: all mice exhibited clinical signs, spongiosis and PrPres). Overall, transmission was more efficient in (1) Swiss than C57Bl/6 mice, (2) PrP^{+/+} than PrP^{+/-} mice, (3) primate than cattle BSE and (4) pellets than supernatant preparations. Among the 8 models exhibiting partial transmission ratios, 29 mice showed clinical neurological signs, of which only one-third (10) had detectable spongiosis and PrPres. The other animals exhibited only PrPres (7), only spongiosis (5) or neither (7).

Conclusion. Our results suggest that clinical neurotoxicity, spongiosis, and accumulation of PrPres are three interconnected but disparate phenomena. Spongiosis and PrPres are specific but not systematic hallmarks of the onset of prion diseases, notably upon first passage of non-adapted prion strains in a new host. These results question the universality of current human diagnostic criteria and the real prevalence of disease linked to BSE exposure.

P.201: Apparent protection of cynomolgus macaques transfused with vCJD infected blood products filtered with prion removal device

Nathalie Lescoutra-Etchegaray,¹ Nina Jaffré,¹

Chryslain Sumian,² Valérie Durand,³ Evelynne Correia,³

Jacqueline Mikol,³ Sophie Luccantoni-Freire,³

Audrey Culeux,¹ Jean-Philippe Deslys,³

and Emmanuel E Comoy³

¹Macopharma; Fontenay-aux-Roses, France; ²Macopharma; Tourcoing, France; ³CEA, Institute of Emerging Diseases and Innovative therapies (iMETI), Division of Prions and related Diseases (SEPIA); Fontenay-aux-Roses, France

Background. A recent analysis of archived appendix samples reveals that 1 in 2,000 individuals in the United Kingdom may carry the infectious prion protein associated with variant Creutzfeldt-Jakob disease. This adjustment of vCJD infection prevalence updates the transfusion-transmitted risk from healthy carriers. Leukoreduction, initially implemented against blood-borne viruses, shows limited efficiency against prions. Therefore, in absence of antemortem diagnostic tests, several prion-removal devices, including the affinity-resin based P-Capt filter, were designed to improve blood transfusion safety.

Study design and methods. We completed the validation of the P-Capt filter, previously assessed in 263K-infected hamster model, with removal of blood-borne infectivity from prion-infected macaques in two independent experiments performed one year apart. At the onset of clinical symptoms, blood was drawn from infected animals. Corresponding red blood cell concentrates were leukoreduced and filtered on P-Capt devices, in combination or with two separate devices. For each study, two groups of three animals were respectively transfused with samples prior or after P-Capt filtration.

Findings. In both studies, recipients of filtered samples are still healthy and devoid of apparent peripheral PrPres 4 to 5 years post transfusion, whereas five out of the six macaques transfused with non-filtered samples developed fatal neurological symptoms 10 to 32 months earlier. Two different fatal neurological disease phenotypes, a classical prion disease or a myelopathic syndrome, were observed in both experiments. The transfusion of 40 mL of whole blood to a macaque induced the development of a classical vCJD, whereas the transfusion of corresponding leucoreduced RBCC induced myelopathy after relatively short incubation periods in the two animals transfused. The transfusion of non-leukoreduced RBCC gave an intermediate pattern with a first animal developing myelopathy at 43 months, and a second one developing a typical vCJD at 48 months post transfusion. These data suggest that depending on the plasma/leukocytes ratio, different neurological phenotypes may arise in this model and that P-Capt filters are able to retain the etiological agents of both types of disease.

P.202: Subtype and regional regulation of prion biomarkers in sporadic Creutzfeldt-Jakob disease

Franc Llorens,^{1,2} Saima Zafar,¹ Belén Ansoleaga,^{2,3} Mohsin Shafiq,¹ Rosi Blanco,^{2,3} Marga Carmona,^{2,3} Ellen Gelpi,⁵ José Antonio del Río,^{3,4,6} Inga Zerr,¹ and Isidre Ferrer^{2,3}

¹Department of Neurology; Clinical Dementia Center and DZNE; University Medical School; Georg-August University; Göttingen, Germany; ²Institute of Neuropathology; IDIBELL-University Hospital Bellvitge; University of Barcelona; Hospitalet de Llobregat, Spain; ³CIBERNED (Network Center for Biomedical Research of Neurodegenerative Diseases); Institute Carlos III; Ministry of Health; Spain; ⁴Molecular and Cellular Neurobiotechnology, Catalan Institute for Bioengineering (IBEC); Parc Científic de Barcelona; Barcelona, Spain; ⁵Neurological Tissue Bank of the Biobanc-Hospital Clínic-Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS); Barcelona, Spain; ⁶Department of Cell Biology, University of Barcelona; Barcelona, Spain

Objective. Creutzfeldt-Jakob disease (CJD) is a rapid progressive neurological disease leading to dementia and death. CJD is caused by misfolding of the cellular prion protein (PrP^C) that converts into the infectious prion form (PrP^{Sc}). Prion biomarkers are altered in the cerebrospinal fluid (CSF) of CJD patients, which is a helpful tool as a molecular diagnostic criterion, but the pathogenic mechanisms underlying these alterations are still unknown. In addition, systematic analysis of brain and CSF

prion biomarker levels and their correlation with neuropathological lesion profiles has not been performed.

Methods. The expression levels of 14-3-3, Tau, phospho-Tau and α -synuclein were measured in the CSF and brain of CJD cases in a subtype- and region-specific manner. In addition, the activity of prion biomarker kinases, the expression levels of CJD hallmarks and the most frequent neuropathological sCJD findings were analysed.

Results. Prion biomarkers levels were increased in the CSF of sCJD patients; however, different correlations between mRNA, total protein and their phosphorylated forms were observed in brain. Finally, the observed downregulated levels of the main Tau kinase, GSK3, in sCJD brain samples may help to explain the differential phospho-Tau/Tau ratios between sCJD and other dementias in the CSF. Importantly, prion biomarker levels do not necessarily correlate with sCJD neuropathological findings.

Interpretation. Our findings indicate that prion biomarkers levels in sCJD tissues and their release into the CSF are differentially regulated following specific modulated responses. These results shed light on the functional role and contribution of prion biomarkers in sCJD pathogenesis.

P.203: Conformational stability of PrP^{Sc} aggregates in sporadic Creutzfeldt-Jakob disease

Maura Cescatti,^{1,2} Daniela Saverioni,^{1,2} Sabina Capellari,^{1,2} and Piero Parchi^{1,2}

¹Dipartimento di Scienze Biomediche e Neuromotorie, Università di Bologna; Bologna, Italy; ²IRCCS, Istituto delle Scienze Neurologiche di Bologna; Bologna, Italy

Introduction. Current evidences indicate that the wide phenotypic variability of prion diseases primarily depends on prion strains with self-perpetuating biological properties that are enciphered in distinct conformations of the misfolded prion protein, PrP^{Sc}. The latter concept, however, is largely based on indirect approaches studying the effects of denaturing agents on the physicochemical properties of PrP^{Sc} aggregates.¹⁻⁴ To further establish whether these indirect methods are able to discriminate among prion strains in humans, we analysed PrP^{Sc} conformational stability across the whole spectrum of sporadic Creutzfeldt-Jakob disease (sCJD) subtypes.⁵

Material and Methods. We studied the effect of guanidine hydrochloride (GndHCl) and heating on PrP^{Sc} aggregates extracted from 50 sCJD-affected brains. Selected samples comprised at least 3 subjects for each pure disease variant, as defined by Parchi et al.⁵ We obtained PrP^{Sc} denaturation curves by plotting the percentage of residual PrP^{Sc} and the percentage of monomeric PrP^{Sc} as a function of GndHCl concentration or temperature, respectively. We then calculated the ED₅₀ for each approach (referred to as [GndHCl]₅₀ and T₅₀) and the percentage of residual PrP^{Sc} at two chosen experimental conditions (1M GndHCl and 35°C).

Results. The denaturation curves obtained after exposing PrP^{Sc} aggregates to increasing GndHCl concentrations showed a

similar profile in all sCJD subtypes. Accordingly, the calculated $[GndHCl]_{50}$, varying from 0,7 M to 1,3 M, did not show any statistically significant difference among the groups analysed. In contrast the effect of temperature on PrP^{Sc} varied significantly according to the sCJD subtype. The calculated T_{50} was lowest for VV1, MM 2T and MM 2C ($T_{50} < 55^{\circ}C$) and highest for MM1 and VV2 ($T_{50} > 80^{\circ}C$), with MV 2K showing intermediate values. A similar trend was obtained by calculating the percentage of detectable PrP^{Sc} signal at 35° rather than the ED_{50} ($< 15\%$ in the “sensitive” groups, $>45\%$ in the “resistant” groups).

Conclusion. Our data suggest that the GndHCl-induced denaturation approach may lack sensitivity in distinguishing prion strains in sCJD. Conversely, the thermostability of PrP^{Sc} aggregates appears correlated with the relative “virulence” of the sCJD strain, given that MM1 and VV2 are the most prevalent phenotypes and those associated with the shortest mean disease duration.

References

1. Kocisko DA, Lansbury PT Jr, Caughey B. Partial unfolding and refolding of scrapie-associated prion protein: evidence for a critical 16-kDa C-terminal domain. *Biochemistry* 1996; 35:13434-42; PMID: 8873612 ; <http://dx.doi.org/10.1021/bi9610562>
2. Safar J, Wille H, Itri V, Groth D, Serban H, Torchia M, Cohen FE, Prusiner SB. Eight prion strains have $PrP(Sc)$ molecules with different conformations. *Nat Med* 1998; 4:1157-65; PMID:9771749; <http://dx.doi.org/10.1038/2654>
3. Pirisinu L, Di Bari M, Marcon S, Vaccari G, D'Agostino C, Fazzi P, Esposito E, Galeno R, Langeveld J, Agrimi U, et al. A new method for the characterization of strain-specific conformational stability of protease-sensitive and protease-resistant PrP . *PLoS One* 2010; 5:e12723; PMID:20856860; <http://dx.doi.org/10.1371/journal.pone.0012723>
4. Bett C, Joshi-Barr S, Lucero M, Trejo M, Liberski P, Kelly JW, Masliah E, Sigurdson CJ. Biochemical properties of highly neuroinvasive prion strains. *PLoS Pathog* 2012; 8:e1002522; PMID:22319450; <http://dx.doi.org/10.1371/journal.ppat.1002522>
5. Parchi P, Giese A, Capellari S, Brown P, Schulz-Schaeffer W, Windl O, Zerr I, Budka H, Kopp N, Piccardo P, et al. Classification of sporadic Creutzfeldt-Jakob disease based on molecular and phenotypic analysis of 300 subjects. *Ann Neurol* 1999; 46:224-33; PMID:10443888; [http://dx.doi.org/10.1002/1531-8249\(199908\)46:2<224::AID-ANA12>3.0.CO;2-W](http://dx.doi.org/10.1002/1531-8249(199908)46:2<224::AID-ANA12>3.0.CO;2-W)

P.204: Creutzfeldt-Jakob disease in the aging United States population

Ryan A Maddox,¹ Marissa K Person,¹ Arialdi M Minino,²
Janis E Blevins,³ Lawrence B Schonberger,¹
and Ermias D Belay¹

¹National Center for Emerging and Zoonotic Infectious Diseases; Centers for Disease Control and Prevention (CDC); Atlanta, GA USA; ²National Center for Health Statistics, CDC; Hyattsville, MD USA; ³National Prion Disease Pathology Surveillance Center (NPDPSC); Case Western Reserve University; Cleveland, OH USA

Introduction. Creutzfeldt-Jakob disease (CJD) predominantly occurs among older individuals. To describe the possible impact of changing demographics in the US population on the occurrence of CJD, we reviewed data from the US census and from national prion disease surveillance.

Methods. Prion disease decedents were identified from the US national multiple cause-of-death data and the National Prion Disease Pathology Surveillance Center database for 2008-2010. Incidence rates were calculated for decedents ≥ 65 years and then

applied to US census population estimates for 2030 to obtain projections of the number of CJD deaths in that year, assuming no advances in treatment or prevention of these diseases.

Results. US census data projects that ≥ 65 -year-olds will increase from 13.1% of the population in 2010 to 20.3% in 2030. The CJD incidence rates for 2008-2010 among decedents in the 65-74, 75-84, and 85+ year age groups were, in cases per million population, 6.5, 7.2, and 3.1, respectively. Applying these incidence rates to US census projections, in 2030 there may be 461 CJD decedents ≥ 65 years of age in the United States, an increase of more than 200 cases compared to the 2008-2010 average for this age group. Of the 461 cases, 251 are projected to be aged 65-74 years, 182 to be aged 75-84 years, and 28 to be aged 85 years or older.

Conclusions. Unless effective treatments for CJD are developed, the aging population in the United States will likely result in an increase in CJD cases due to its higher incidence among older adults. The increase in cases could impact infection control policies and health care costs, among other factors.

P.205: Prion protein aggregation triggered by neutrophil extracellular traps (NETs)

Daniel M Anjos,¹ Estefania P Azevedo,¹ Tuane C.R.G Vieira,¹
Debora Foguel,¹ Elvira M Saraiva,² and Jerson L Silva¹

¹Instituto de Bioquímica Médica, IBqM-UFRJ; RJ Brazil; ²Instituto de Microbiologia, IMPPG-UFRJ; RJ Brazil

Prion diseases are fatal neurodegenerative protein-misfolding diseases related to a protein known as prion protein (PrP). The constitutive cellular isoform of PrP (PrP^C), present in the cell surface, can be converted into its abnormal, β -sheet-rich isoform (PrP^{Sc}) that undergoes aggregation. PrP^{Sc} can be transmissible and infectious. Evidences suggest that adjuvant factors may play a role in the conversion process. Our group previously reported that DNA can convert PrP^C into a β -sheet conformation leading to its aggregation.

Innate immune system cells have been proven to play critical roles in the incunabular pathogenic events of prion diseases and may be involved in its progression. Following peripheral exposure, the replication of prions within the lymphoid tissue has been shown to be important for the efficient spread of the disease to the brain.

Neutrophil extracellular traps (NETs) are large DNA networks decorated with histones and granule proteins that trap and kill pathogens, being extruded by activated neutrophils in response to several stimuli. NETs were reported of being released in lymphoid tissues. Furthermore, NETs were recently found in association with amyloid fibrils in tissues of patients with other protein-misfolding diseases and these fibrils triggered the release of NETs.¹ Regarding such observations and considering the large quantity of DNA in NETs we asked if it could induce PrP^C aggregation in vitro. Murine recombinant PrP23-231 was added into the supernatants from activated human neutrophils containing

NETs. Aggregation was measured by light scattering and electron microscopy. The results show NETs dose-dependently triggering an instantaneous PrP^C aggregation. It gradually declined remaining detectable after 1 hour. The aggregates showed amorphous morphology and absence of fibrils. NETs previous treatment with DNase I inhibited PrP^C aggregation, pointing out the importance of the DNA integrity for its effect. Our results show that NETs are able to trigger stable PrP^C aggregation and that its DNA networks are crucial for this process. Differences in NETs protein composition among different donors seems to be related to distinct aggregation features.

Our data suggest that NETs are an intriguing factor that must be appraised in the studies concerning prion propagation mechanisms.

References

1. Azevedo EP, Guimarães-Costa AB, Torezani GS, Braga CA, Palhano FL, Kelly JW, Saraiva EM, Foguel D. Amyloid fibrils trigger the release of neutrophil extracellular traps (NETs), causing fibril fragmentation by NET-associated elastase. *J Biol Chem* 2012; 287:37206-18; PMID:22918834; <http://dx.doi.org/10.1074/jbc.M112.369942>

P.206: A novel de novo insertional mutation in the prion protein gene characterized by slowly progressive atypical dementia: Neuropathological and biochemical findings

Gianfranco Puoti,¹ Dario Saracino,¹ Cinzia Coppola,¹ and Ignazio Cali^{1,2}

¹Division of Neurology; Department of Clinical and Experimental Medicine; Second University of Naples; Naples, Italy; ²National Prion Disease Pathology Surveillance Center (NPDPSC); Case Western Reserve University; School of Medicine; Cleveland, OH USA.

Introduction. Like the sporadic form of Creutzfeldt-Jakob disease (sCJD), the inherited or genetically transmitted prion diseases can be phenotypically heterogeneous. Recently, we described the peculiar clinical features of a novel de novo seven-octapeptide repeat insertional mutation (7-OPRI) in the prion protein (PrP) gene of a 27 year old man. The clinical phenotype was characterized by the very long disease duration, psychiatric symptoms and atypical dementia at onset related to marked parietal atrophy.

Materials and Methods. In the present study we carried out a detailed description of the neuropathology and molecular features of the scrapie PrP (PrP^{Sc}) in the autopsied brain of this genetic CJD case.

Results. Neuropathology consisted of focal spongiform degeneration and severe diffuse astrogliosis mainly detected in the parietal cortex. Immunohistochemistry showed clusters of scattered PrP aggregates in a background of synaptic PrP in the parietal cortex and hippocampus, as well as single or multicore PrP plaques in the subiculum; PrP patches running perpendicularly to the pial surface of the cerebellar molecular layer, were also present. The molecular study confirmed the presence of the proteinase-K resistant PrP^{Sc} (PrP^{res}) type 1 in the cerebral cortex and

the co-occurrence of PrP^{res} types 1 and 2 limited to the cerebellum. At variance from sCJD, the present case was characterized by (i) different ratios of soluble and insoluble PrP, (ii) a different profile of the diglycosylated insoluble PrP^{Sc}, (iii) presence of a ~39-40 kDa PrP fragment, and iv) greater levels of C-terminal low molecular weight PrP^{res} fragments.

Conclusions. Along with the atypical clinical picture, the features above reported define a peculiar neuropathological and biochemical phenotype associated with the novel *PRNP* haplotype of the PrP gene, further expanding the spectrum of genotype-phenotype correlations in inherited prion diseases.

Acknowledgments. Supported by P01AG 14359, CDC UR8/CCU515004 and Charles S. Britton Fund.

P.207: RT-QuIC detection of PrP^{sCJD} spiked into human urine

Sarah Vascellari,¹ Andrew G Hughson,² Franco Cardone,³ Maurizio Pocchiari,³ Byron Caughey,² Christina D Orrù,^{1,2} and Alessandra Pani¹

¹Department of Biomedical Sciences; University of Cagliari; Cagliari, Italy; ²Laboratory of Persistent Viral Diseases; Rocky Mountain Laboratories; National Institute of Allergy and Infectious Diseases; National Institutes of Health; Hamilton, MT USA;

³Department of Cell Biology and Neurosciences; Istituto Superiore di Sanità; Rome, Italy

Keywords: RT-QuIC, recombinant PrP^{Scn}, diagnosis, sCJD, urine

Management of Transmissible Spongiform Encephalopathies (TSE) requires the ability to sensitively detect prions in biological tissues. The real time quaking induced conversion (RT-QuIC) assay is a rapid and highly sensitive assay for prion seeding activity that has been applied to several host species and sample types, including plasma,^{1,2} cerebral spinal fluid (CSF),^{3,4,5,6,7,8} nasal secretions,^{9,10} saliva¹¹ and urine.¹² Here we describe the use of this assay for the detection of brain-derived human sCJD seeding activity spiked into urine.

Our studies show that the RT-QuIC detects down to ~10⁻⁷ sCJD final brain tissue dilutions in 0.5mL of urine with no spontaneous (unseeded) conversion of the prion protein substrate within an 80h reaction. To further improve the sensitivity of the assay we introduced a PrP^{sCJD} concentration step by phosphotungstic acid (PTA) precipitating PrP^{sCJD} spiked into urine. Our results show a thousand-fold increase in sensitivity following PTA-precipitation in a range of PrP^{sCJD} spiked urine volumes. These preliminary findings suggest that the RT-QuIC allows detection of brain-derived PrP^{sCJD} diluted into urine and show promise for the application of the assay to the detection of endogenous prion seeding activity in urine from sCJD patients.

References

1. Orrù CD, Wilham JM, Raymond LD, Kuhn F, Schroeder B, Raeber AJ, Caughey B. Prion disease blood test using immunoprecipitation and improved quaking-induced conversion. *mBio* 2011; 2:e00078-11; PMID: 21558432; <http://dx.doi.org/10.1128/mBio.00078-11>

2. Vascellari S, Orrù CD, Hughson AG, King D, Barron R, Wilham JM, Baron GS, Race B, Pani A, Caughey B. Prion seeding activities of mouse scrapie strains with divergent PrP^{Sc} protease sensitivities and amyloid plaque content using RT-QuIC and eQuIC. *PLoS One* 2012; 7:e48969; PMID:23139828; <http://dx.doi.org/10.1371/journal.pone.0048969>
3. Atarashi R, Satoh K, Sano K, Fuse T, Yamaguchi N, Ishibashi D, Matsubara T, Nakagaki T, Yamanaka H, Shirabe S, et al. Ultrasensitive human prion detection in cerebrospinal fluid by real-time quaking-induced conversion. *Nat Med* 2011; 17:175-8; PMID:21278748; <http://dx.doi.org/10.1038/nm.2294>
4. Wilham JM, Orrù CD, Bessen RA, Atarashi R, Sano K, Race B, Meade-White KD, Taubner LM, Timmes A, Caughey B. Rapid end-point quantitation of prion seeding activity with sensitivity comparable to bioassays. *PLoS Pathog* 2010; 6:e1001217; PMID:21152012; <http://dx.doi.org/10.1371/journal.ppat.1001217>
5. Orrù CD, Hughson AG, Race B, Raymond GJ, Caughey B. Time course of prion seeding activity in cerebrospinal fluid of scrapie-infected hamsters after intratongue and intracerebral inoculations. *J Clin Microbiol* 2012; 50:1464-6; PMID:22238438; <http://dx.doi.org/10.1128/JCM.06099-11>
6. McGuire LI, Peden AH, Orrù CD, Wilham JM, Appleford NE, Mallinson G, Andrews M, Head MW, Caughey B, Will RG, et al. Real time quaking-induced conversion analysis of cerebrospinal fluid in sporadic Creutzfeldt-Jakob disease. *Ann Neurol* 2012; 72:278-85; PMID:22926858; <http://dx.doi.org/10.1002/ana.23589>
7. Haley NJ, Van de Motter A, Carver S, Henderson D, Davenport K, Seelig DM, Mathiason C, Hoover E. Prion-seeding activity in cerebrospinal fluid of deer with chronic wasting disease. *PLoS One* 2013; 25;8(11):e81488; PMID: 24282599; <http://dx.doi.org/10.1371/journal.pone.0081488>
8. Sano K, Satoh K, Atarashi R, Takashima H, Iwasaki Y, Yoshida M, Sanjo N, Murai H, Mizusawa H, Schmitz M, et al. Early detection of abnormal prion protein in genetic human prion diseases now possible using real-time QUIC assay. *PLoS One* 2013; 8:e54915; PMID:23372790; <http://dx.doi.org/10.1371/journal.pone.0054915>
9. Bessen RA, Shearin H, Martinka S, Boharski R, Lowe D, Wilham JM, Caughey B, Wiley JA. Prion shedding from olfactory neurons into nasal secretions. *PLoS Pathog* 2010; 6:e1000837; PMID:20419120; <http://dx.doi.org/10.1371/journal.ppat.1000837>
10. Bessen RA, Wilham JM, Lowe D, Watschke CP, Shearin H, Martinka S, Caughey B, Wiley JA. Accelerated shedding of prions following damage to the olfactory epithelium. *J Virol* 2012; 86:1777-88; PMID:22130543; <http://dx.doi.org/10.1128/JVI.06626-11>
11. Henderson DM, Manca M, Haley NJ, Denkers ND, Nalls AV, Mathiason CK, Caughey B, Hoover EA. Rapid antemortem detection of CWD prions in deer saliva. *PLoS One* 2013; 8:e74377; PMID:24040235; <http://dx.doi.org/10.1371/journal.pone.0074377>
12. John TR, Schätzl HM, Gilch S. Early detection of chronic wasting disease prions in urine of pre-symptomatic deer by real-time quaking-induced conversion assay. *Prion* 2013; 7:253-8; PMID:23764839; <http://dx.doi.org/10.4161/pri.24430>

P.208: Optimization of the Real Time Quaking Induced Conversion (RTQUIC) reaction adopting different recombinant prion proteins

Gabriele Piconi,¹ Anna Poleggi,¹ Gabriele Giachin,² Michele Equestre,¹ Giovanna Avella,³ Beatrice Vallone,³ Franco Cardone,¹ Anna Ladogana,¹ Giuseppe Legname,² and Maurizio Pocchiari¹

¹Istituto Superiore di Sanità, Department of Cell Biology and Neurosciences; Rome, Italy; ²Department of Neuroscience, Laboratory of Prion Biology, Scuola Internazionale Superiore di Studi Avanzati (SISSA); Trieste, Italy; ³Istituto Pasteur-Fondazione Cenci Bolognietti and Istituto di Biologia e Patologia Molecolari del CNR, Dipartimento di Scienze Biochimiche "A. Rossi Fanelli", Sapienza Università di Roma; Rome, Italy

Early diagnosis of prion diseases is crucial for a better control of iatrogenic transmission, for the evaluation of disease progression as well as for the design of clinical trials. In the last decade a number of in-vitro techniques able to amplify and detect small amount of prions like those present in the cerebrospinal fluid (CSF) have been published. Among them, the Real Time Quaking Induced Conversion reaction (RTQUIC) has shown

the ability to discriminate between human sporadic Creutzfeldt-Jakob disease (sCJD) CSF and non-sCJD CSF samples with an unprecedented sensitivity and specificity paving the way to become a method for ante-mortem diagnosis.¹⁻⁴ This new methodology employs bacterially-expressed recombinant prion protein (rPrP) as substrate. The rPrP substrate is converted into a Thioflavin T (ThT) reactive form during the reaction only if prions are present in the sample under analysis. Precise reaction conditions and adequate reagents are required to obtain the highest efficiency in the conversion of rPrP into an amyloid-like structure. In this work we analysed the contribution of some of the main parameters affecting the conversion.

We show that RTQUIC reaction can be optimized to employ different recombinant substrate for the detection of prions in human CSF and 263K hamster brain homogenate by tuning reaction parameters such as plate shaking intensity, sodium chloride or substrate concentration. Moreover, in agreement with Cheng and colleagues⁵ we also observed that recombinant protein preparation protocol is crucial for the RTQUIC assay. Indeed, we observed that the same recombinant prion protein produced following two different protocols determines different results in RTQUIC. In conclusion, our findings confirm the potential of RTQUIC as diagnostic test for prion disease but, at the same time, highlight the need for a better comprehension of the biophysical aspects influencing the RTQUIC reaction.

References

1. Atarashi R, Satoh K, Sano K, Fuse T, Yamaguchi N, Ishibashi D, Matsubara T, Nakagaki T, Yamanaka H, Shirabe S, et al. Ultrasensitive human prion detection in cerebrospinal fluid by real-time quaking-induced conversion. *Nat Med* 2011; 17:175-8; PMID:21278748; <http://dx.doi.org/10.1038/nm.2294>
2. Wilham JM, Orrù CD, Bessen RA, Atarashi R, Sano K, Race B, Meade-White KD, Taubner LM, Timmes A, Caughey B. Rapid end-point quantitation of prion seeding activity with sensitivity comparable to bioassays. *PLoS Pathog* 2010; 6:e1001217; PMID:21152012; <http://dx.doi.org/10.1371/journal.ppat.1001217>
3. Peden AH, McGuire LI, Appleford NE, Mallinson G, Wilham JM, Orrù CD, Caughey B, Ironside JW, Knight RS, Will RG, et al. Sensitive and specific detection of sporadic Creutzfeldt-Jakob disease brain prion protein using real-time quaking-induced conversion. *J Gen Virol* 2012; 93:438-49; PMID:22031526; <http://dx.doi.org/10.1099/vir.0.033365-0>
4. McGuire LI, Peden AH, Orrù CD, Wilham JM, Appleford NE, Mallinson G, Andrews M, Head MW, Caughey B, Will RG, et al. Real time quaking-induced conversion analysis of cerebrospinal fluid in sporadic Creutzfeldt-Jakob disease. *Ann Neurol* 2012; 72:278-85; PMID:22926858; <http://dx.doi.org/10.1002/ana.23589>
5. Cheng K, Sloan A, Avery KM, Coulthart M, Carpenter M, Knox JD. Exploring physical and chemical factors influencing the properties of recombinant prion protein and the real-time quaking-induced conversion (RT-QuIC) assay. *PLoS One* 2014; 9:e84812; PMID:24404191; <http://dx.doi.org/10.1371/journal.pone.0084812>

P.209: Diagnostic value of surrogate cerebrospinal fluid biomarkers for the clinical diagnosis of Creutzfeldt-Jakob disease: A 10-year review from a CJD referral laboratory

Ilaria Poggiolini,^{1,2} Sabina Capellari,^{1,2} Anna Ladogana,³
Lynne I McGuire,⁴ Alison JE Green,⁴ Maurizio Pocchiarini,³
and Piero Parchi^{1,2}

¹Dipartimento di Scienze Biomediche e Neuromotorie, Università di Bologna; Bologna Italy; ²IRCCS, Istituto delle Scienze Neurologiche di Bologna; Bologna, Italy; ³Department of Cell Biology and Neurosciences, Istituto Superiore di Sanità; Rome, Italy; ⁴The National CJD Research & Surveillance Unit, University of Edinburgh; Edinburgh, Scotland, UK

Background. Differential diagnosis of Creutzfeldt–Jakob disease (CJD) versus other, sometimes treatable, neurological disorders is challenging, owing to the wide phenotypic heterogeneity of the disease and to uncertainty about the value of recommended diagnostic assays. To contribute to the definition of the diagnostic utility of surrogate cerebrospinal fluid (CSF) biomarkers, such as protein 14-3-3 and total tau (T-Tau), we have analyzed the results obtained in 500 sequentially recruited Italian patients with clinically suspected CJD.

Methods. We measured CSF concentrations of T-Tau, P₁₈₁-Tau and Aβ₁₋₄₂ using the INNOTEST ELISA assays, and detected the 14-3-3 protein by Western blot. We chose a cut-off value of 1300 pg/mL for T-Tau, and rated 14-3-3 protein immunoreactivity as positive or negative based on the densitometric comparison with a control showing “borderline” levels of the protein. We obtained the final diagnosis either by neuropathological examination or clinical follow-up (evolution of symptoms for at least 6 months, results of cerebral MRI, EEG and other laboratory investigations). Finally, we used in vitro PrP^{Sc} amplification by RT-QuIC to further corroborate the diagnosis of CJD in the group of patients lacking post-mortem confirmation, classified as possible or probable CJD based on adequate clinical follow-up.

Results. 239 patients (174 definite, 65 probable) received a diagnosis of prion disease, 241 had an alternative diagnosis, while the remaining 20 subjects lacked sufficient clinical information for a diagnostic categorization. 14-3-3 assay produced an overall test sensitivity of 87% and a specificity of 88%, whereas T-Tau test had a sensitivity of 90% and a specificity of 93%. In sporadic (s) CJD, the sensitivity of both 14-3-3 and T-Tau assays varied significantly according to the disease subtype. It was highest in VV2 and MM1, lowest in MM 2T, and intermediate in MM 2K and MM 2C. Both VV2 and MV 2K subjects showed increased CSF levels of P₁₈₁-Tau when compared to sCJD MM1, MM 2C, and MM2T, genetic CJD and normal age-matched controls. Finally, subjects with probable or definite CJD showed reduced Aβ₁₋₄₂ CSF levels below normal range in about 55% of cases.

Conclusions. CSF 14-3-3 and T-Tau proteins are useful diagnostic markers for the most common CJD subtypes (MM1 and VV2) even in a non-selected clinical population. In this study, T-Tau assay performed better than 14-3-3 protein mainly because of a higher specificity. Increased P₁₈₁-Tau in VV2 and MV 2K

subjects suggests that the neurodegeneration associated with the V2 prion strain comprises a secondary tauopathy.

P.210: Further characterization of PK-resistant PrP^{Sc} core fragments in sporadic CJD brains carrying VV or MV at PRNP codon 129

Daniela Saverioni,^{1,2} Sabina Capellari,^{1,2} Atsushi Kobayashi,³
Tetsuyuki Kitamoto,³ and Piero Parchi^{1,2}

¹Dipartimento di Scienze Biomediche e Neuromotorie, Università di Bologna; Bologna, Italy; ²IRCCS, Istituto delle Scienze Neurologiche di Bologna; Bologna, Italy; ³Division of Neurological Science, Tohoku University Graduate School of Medicine; Sendai, Japan

The recognition that the wide phenotypic variability of sporadic Creutzfeldt–Jakob disease (sCJD) largely correlate with codon 129 genotype and size of PK-resistant core (PrP-res) of PrP^{Sc}, has allowed, for the first time, a molecular classification of the disease. Current limitations of this approach, however, include the incomplete discrimination between PrP-res fragments of the same size correlating with distinct clinico-pathological phenotypes or having a different origin and significance. The development of PrP-res types 1 or 2 selective antibodies, for example, led to the discovery of fragments sharing the same migration profile of “bona-fide” types and 1 and 2, but displaying divergent physicochemical properties. To further contribute to the characterization of CJD-associated PrP-res fragments and to the discrimination between strain-specific and secondary “type-like” fragments, we studied PrP^{Sc} properties in sCJD brains harbouring VV or MV at PRNP codon 129, using both standard (SAF60, 3F4) and PrP-res type “selective” (12B2, T2) antibodies. To this aim brain tissue from patients affected by sCJD VV1, VV2, VV1+2, MV 2K, MV 2C, MV 2C+2K, and MM 2C was homogenized and PK digested at pH 8 at various enzyme concentrations.

Our preliminary results indicate that:

(1) Type 2-selective antibody T2 consistently recognizes traces of a 19 kDa PrP-res fragment in VV1 brain homogenates. However, the degree of PK-resistance of this fragment is significantly lower than that of PrP^{Sc} type 2 from sCJD VV2 and VV1+2. Moreover, the 19 kDa fragment recognized by T2 in cerebellum is even less abundant than in frontal cortex, which is at variance with the typical PrP^{Sc} deposition pattern in sCJD VV2. It is concluded that the 19 kDa recognized by T2 likely represents a secondary product not related to the V2 strain.

(2) In sCJD MV 2K, the PK-resistance of type 2 (19 kDa) fragment is comparable to that of type 2 fragments from VV2 brains, but significantly higher than that of the 20 kDa associated fragment. These results support the hypothesis that sCJD VV2 and MV 2K are caused by the same strain, whereas a host genotype effect is the most likely explanation for the different properties of the 20 kDa associated fragment.

(3) There is no difference in PrP^{Sc} type 2 PK-resistance between sCJD MM 2C and MV 2C. Thus, at variance with the

MV 2K subtype, the codon 129 heterozygous genotype seems to have no influence on the PrP^{Sc} aggregate associated with this strain.

P.211: CJD subtype specific interactive role of ADP ribosylation factor-1

Saima Zafar,¹ Abdul R Asif,² Amandeep Singh Arora,¹
Matthias Schmitz,¹ Franc Llorens,¹ Isidre Ferrer^{3,4}
and Inga Zerl¹

¹Clinical Dementia Center; Department of Neurology and Psychiatry; University Medical Center Goettingen (UMG); Goettingen, Germany; ²Department of Clinical Chemistry; University Medical Center Goettingen (UMG); Goettingen, Germany; ³Institute of Neuropathology; IDIBELL-University Hospital Bellvitge; University of Barcelona; Hospitalet de Llobregat; Barcelona, Spain; ⁴CIBERNED (Network Center for Biomedical Research of Neurodegenerative Diseases); Institute Carlos III; Ministry of Health; Madrid, Spain

Keywords: Prion protein, Interacting protein, Arf1, BFA, intracellular PrP^C distribution, Creutzfeldt-Jacob disease (CJD), MM1, VV2, frontal cortex, cerebellum

Small GTPases of the ADP ribosylation factor (Arf) family mainly activate the formation of coated carrier vesicles and primarily localize to the Golgi apparatus. The activation of membrane vesicle formation occurs after the replacement of Arf-bound GDP with GTP. We recently showed that class I Arf1 interacts with cellular form of prion protein PrP^C which is GPI-anchored glycoprotein and required for the conversion of PrP^C to the transmissible spongiform encephalopathy-associated protease-resistant isoform. Several recent studies also discussed about the missing link between ER and Golgi for proper folding in association with cytoskeleton network, and glycosylation of PrP^C but still not yet fully understood. In the present study we characterized the interactive role of Arf1 during PrP^C intracellular distribution under physiological condition and in prion (Creutzfeldt-Jacob) disease. PrP^C interaction with Arf1 was investigated after transiently expressing PrP^C in mice hippocampal prion protein (PrP) gene-deficient (HpL3-4) cells and in primary neuronal cultures of C57Bl6 PrP^C knockout mice [PrP(-/-)]. Interaction of Arf1 was confirmed by using confocal laser scanning microscopy and reverse co-immunoprecipitation. Pearson's correlation coefficient also showed partial but significant co-localization of Arf1 with PrP^C. Treatment with brefeldin A, an intracellular Arf1 protein transport inhibitor, resulted in downregulation of PrP^C expression and redistribution of PrP^C into the cytosol. Furthermore, first time we examined Arf1 expression in the brain (frontal cortex and cerebellum) of prion (Creutzfeldt-Jacob) disease subtype (MM1 and VV2) cases (15 MM1, 15 VV2 and 15 age matched controls). In frontal cortex Arf1 expression was significantly reduced only in VV2 subtype, but in cerebellum Arf1 expression was significantly reduced in both subtypes as compared with controls. These observations indicate potential functional interaction of Arf1 with PrP^C and that expressional alteration of Arf1

in Creutzfeldt-Jacob disease subtype manner could possibly be the missing link which probably will influence the disease progression or formation and distribution of misfolding proteins.

Acknowledgments. Supported by EU grant "JPND"

P.212: Molecular mechanism of TNT-mediated transfer of alpha-synuclein aggregates in neuronal cells

Saïda Abounit,¹ Luc Bousset,² Ronald Melki,²
Alexandre Dufour,³ and Chiara Zurzolo¹

¹Unité Trafic Membranaire et Pathogénèse, Pasteur Institute; Paris, France; ²Repliement des protéines in vivo et maladies conformationnelles, LEBS; Gif-sur-Yvette, France; ³Analyse d'images quantitative, Pasteur Institute; Paris, France

Keywords: prion-like proteins, alpha-synuclein, Tunneling Nanotubes (TNTs)

Neurodegenerative diseases (NDs) such as Prion disease, Alzheimer's disease (AD), Parkinson's disease (PD) and Huntington's disease (HD) are part of a large group of protein misfolding disorders (PMDs) characterized by the progressive accumulation and spreading of protein aggregates. The best-characterized PMD is the Prion disease, which is caused by the conversion of the normal form of the prion protein (PrP^C), to a misfolded form (PrP^{Sc}) through a process called "template conformation change". Like in Prion disease, misfolded forms of tau and alpha-synuclein (associated respectively with AD and PD) can be transmitted experimentally in cellular and animal models. They subsequently act as "seeds" by recruiting the endogenous protein into aggregates (seeding process). However, the mechanism of intercellular transfer is still obscure. We have recently described a novel mechanism for the spreading of PrP^{Sc} and huntingtin aggregates through tunneling nanotubes (TNTs). TNTs are fine actin-based protrusions connecting sparse cells in culture. They represent a novel mechanism of cell-to-cell communication.¹ We showed that TNTs mediate the transfer of PrP^{Sc} between infected and naïve mouse neuronal cells and between bone-marrow dendritic cells and primary neurons.² More recently we demonstrated that aggregates of mutant huntingtin hijack TNTs in neuronal cells, therefore we propose that TNTs can represent an efficient route for protein aggregate spreading.³ To validate this hypothesis we are currently analysing whether and to which extent alpha-synuclein transfers between cells through TNTs. Interestingly, we found that both alpha-synuclein fibrillar and oligomeric aggregates transfer between neuronal cells and that this transfer is dependent on cell-to-cell contact. Furthermore we have shown using microscopy that alpha-synuclein aggregates are found within TNTs between donor and acceptor cell populations. To further investigate the role of TNTs in this transfer, we overexpressed Myosin10 (molecular motor previously shown by the laboratory to induce the formation of functional TNTs in neuronal cells)⁴ in the donor cell population and found that the rate of transfer of synuclein aggregates was highly increased for

both fibrils and oligomers. Taken together these results prompted us to investigate more in details the molecular mechanism of TNT-mediated alpha-synuclein aggregate transfer in neuronal cells. To do so, we started to identify the sub-cellular localization of alpha-synuclein aggregates; the ones present in donor cells and the ones that have been transferred from donor to acceptor cells. So far, we have found transferred alpha-synuclein fibrils colocalizing with Lamp-1 positive vesicles suggesting that part of alpha-synuclein fibrils transfer via TNTs in lysosomes.

References

1. Abounit S, Zurzolo C. Wiring through tunneling nanotubes--from electrical signals to organelle transfer. *J Cell Sci* 2011; 125:1089-98; PMID: 22399801; <http://dx.doi.org/10.1242/jcs.083279>
2. Gousset K, Schiff E, Langevin C, Marijanovic Z, Caputo A, Browman DT, Chenouard N, de Chaumont F, Martino A, Enninga J, et al. Prions hijack tunnelling nanotubes for intercellular spread. *Nat Cell Biol* 2009; 11:328-36; PMID:19198598; <http://dx.doi.org/10.1038/ncb1841>
3. Costanzo M, Abounit S, Marzo L, Danckaert A, Chamoun Z, Roux P, Zurzolo C. Transfer of polyglutamine aggregates in neuronal cells occurs in tunneling nanotubes. *J Cell Sci* 2013; 126:3678-85; PMID: 23781027; <http://dx.doi.org/10.1242/jcs.126086>
4. Gousset K, Marzo L, Commere PH, Zurzolo C. Myo10 is a key regulator of TNT formation in neuronal cells. *J Cell Sci* 2014; 126:4424-35; PMID: 23886947; <http://dx.doi.org/10.1242/jcs.129239>

P.213: Prion disease associated with diarrhea and autonomic neuropathy: Phenotypic and genetic characterization of an Italian family

Sabina Capellari,^{1,2} Piero Parchi,^{1,2} Patrizia Corrado,¹
Federica Provini,^{1,2} Giovanna Calandra Buonauro,^{1,2}
Giulia Pierangeli,^{1,2} Anna Bartoletti Stella,¹
Vincenzo Donadio,² Raffaele Lodi,¹ Caterina Tonon,¹
and Pietro Cortelli^{1,2}

¹Department of Biomedical and Neuromotor Science, Alma Mater Studiorum, University of Bologna; ²IRCCS Istituto delle Scienze Neurologiche di Bologna, Bologna Italy

Introduction. Human prion diseases typically manifest as rapidly progressive neurological syndromes secondary to CNS involvement. Recently, however, a largely peripheral phenotype characterized by chronic diarrhea and autonomic neuropathy was linked to a Y163X truncating mutation in the prion protein gene (*PRNP*).¹ This peculiar phenotype is thought to be driven by the lack of the glycosylphosphatidylinositol moiety anchoring the prion protein to the cell membrane, which favors the spread of the pathological protein through the interstitial fluid and perivascular areas leading to CAA and/or systemic amyloidosis.²

Materials and Methods. We performed a comprehensive phenotypic and diagnostic evaluation, including genealogical, neuropsychological, neurophysiological, neuroimaging, molecular genetics, and biochemical studies in a patient affected by familial form of chronic, disabling dysautonomia.

Results. Genetic analysis discovered a small 9 base pair duplication in the *PRNP* ORF resulting in a stop codon (Y163X). The index patient, his brother and his father developed a slowly progressive disease in the fifth decade. Urinary dysfunction with

orthostatic hypotension and a disabling diarrhea were the dominant clinical signs, while cognitive decline manifested in one subject at a late disease stage. EMG showed a mixed, sensory-motor, mainly axonal, polyneuropathy affecting the inferior limbs, autonomic studies discovered a mainly peripheral dysautonomia, while skin innervations studies demonstrated a total loss of cholinergic and noradrenergic fibres mainly affecting the distal sites. Polysomnography showed a reduction of both total sleep time and sleep efficiency and, late in the disease course, a marked reduction of NREM sleep figures associated with an increase of slow wave sleep. Cerebral RMN FLAIR and T2- images showed a mild diffuse atrophy sparing the occipital lobes, and multiple hyperintense foci in subcortical and periventricular white matter and in the pons. CSF analysis demonstrated increased total protein count with elevated levels of IgG and albumin and elevated T-Tau and 14-3-3 protein levels.

Discussion. The present report confirms that a peripheral phenotype dominated by diarrhea and autonomic neuropathy can be caused by a genetic form of prion disease. Compared to the family previously reported,¹ our patients had a milder form and carried a distinct genetic defect, though leading to the same *PRNP* 163 stop codon. Taken together the two reports widen the spectrum of prion amyloidosis linked to truncating mutations in *PRNP*.

References

1. Mead S, Gandhi S, Beck J, Caine D, Gajulapalli D, Carswell C, Hyare H, Joiner S, Ayling H, Lashley T, et al. A novel prion disease associated with diarrhea and autonomic neuropathy. *N Engl J Med* 2013; 369:1904-14; PMID:24224623; <http://dx.doi.org/10.1056/NEJMoa1214747>
2. Rangel A, Race B, Phillips K, Striebel J, Kurtz N, Chesebro B. Distinct patterns of spread of prion infection in brains of mice expressing anchorless or anchored forms of prion protein. *Acta Neuropathol Commun* 2014; 2:8; PMID:24447368; <http://dx.doi.org/10.1186/2051-5960-2-8>

P.214: Psychosocial issues of at-risk individuals of genetic prion disease

Chieko Tamura^{1,2}

¹Medical Information and Genetic Counseling Office; Fetal Medicine Clinic Tokyo; Tokyo, Japan; ²Genetic Counseling Clinic; Juntendo University Hospital; Tokyo, Japan

Keywords: genetic prion disease, psychosocial issues, psychological support, genetic counseling

Genetic Creutzfeldt-Jakob disease, Gerstmann-Sträussler-Scheinker syndrome, and Fatal Familial Insomnia are known as the genetic form of prion disease, although penetrance varies depending on the conditions and mutation sites. According to the Japanese prion disease surveillance program data, genetic cases account for around 15% of total prion disease patients. We have conducted genetic counseling sessions with family members of these patients in Japan, including at-risk individuals who may or may not have inherited mutations from the patients. Through our experiences, there seem to be some psychosocial issues that at-risk individuals tend to have.

One of the major issues is how to break the news that the particular patient's prion disease is genetic to at-risk individuals

in the family. As it is not always easy for medical staff to contact many relatives of the patients, physicians sometimes need to tell that the patients' prion disease is genetic to the spouses of the patients, who are not blood-relatives, and the spouses sometimes do not deliver the news to their children and patients' blood relatives. Or, even if children or siblings of the patients were told, they may need help to distribute the news to other relatives. Whichever spouses or blood-relatives, family members of patients of genetic prion disease tend to have difficulties to discuss this matter with other relatives in the families, as the news is devastating. Not only professional psychosocial help, but also peer support through the family support group may be helpful, in the future.

After getting the news, at-risk individuals have to live with the risk of developing prion disease. This situation often affects the life decisions along various life events of these people. They may also want to consider pre-symptomatic predictive genetic testing, as well as prenatal and/or pre-implantation genetic diagnosis for future offspring, which is mostly not available in Japan, currently.

With at-risk individuals, medical professionals may need to discuss the way of precaution of possible future prion transmission from at-risk individuals, although little is known about the timing and the extent to which at-risk individuals should be alerted. Furthermore, there are not enough data on penetrance rate of each mutations. Further studies are needed for these aspects.

Overall, it is important to understand the psychosocial situations and problems of at-risk individuals of genetic prion disease, and how to support these people needs to be considered.

P.215: Detection and quantification of low prion protein levels in the peripheral tissues by capillary electrophoresis

Amandeep Singh Arora, Matthias Schmitz, Saima Zafar, and Inga Zerr

UMG Göttingen, Prion Research Group; Göttingen, Germany

The cellular prion protein (PrP^C) is a glycosylated membrane protein. Its misfolding form is involved in a set of neurodegenerative diseases in humans as well as in animals. PrP^C is high abundantly expressed in brain tissue with comparatively very less expression in peripheral tissues. Emergence of liver as a prion infectivity or PrP^{Sc} positive organ^{1,2,3} with contradictory undetectable or diminish levels of PrP^C has been described in most of the studies.^{4,5,6} Detection and accurate quantification of PrP^C is important to understand its biological function and involvement of peripheral organs in disease mechanism.

In the present study we established a highly sensitive method to detect and quantify minimal amount of PrP^C in mice liver using fluorescent peptides raised against different regions of PrP^C. In this test, PrP^C is firstly allowed to bind on sequence specific antibody coupled with protein A magnetic beads. Fluorescent

peptide sequence homolog to PrP^C with known concentration is incubated with PrP-antibody-bead complex. Peptide binding to antibody is partially blocked by specifically and quantitatively bound PrP^C. Unbound peptide from supernatant corresponds to amount of PrP^C compared to controls. Fluorescent peptide is quantified by Laser-Induced Fluorescence (LIF) module of Capillary electrophoresis.

In conclusion, this method seems to be a promising approach to quantify subtle changes in PrP^C levels in the peripheral tissues with future prospective to PrP^{Sc} detection coupled with PK digestion.

References

1. Mead S, Gandhi S, Beck J, Caine D, Gajulapalli D, Carswell C, Hyare H, Joiner S, Ayling H, Lashley T, et al. A novel prion disease associated with diarrhea and autonomic neuropathy. *N Engl J Med* 2013; 369:1904-14; PMID:24224623; <http://dx.doi.org/10.1056/NEJMoa1214747>
2. Everest SJ, Ramsay AM, Chaplin MJ, Everitt S, Stack MJ, Neale MH, Jeffrey M, Moore SJ, Bellworthy SJ, Terry LA. Detection and localisation of PrP(Sc) in the liver of sheep infected with scrapie and bovine spongiform encephalopathy. *PLoS One* 2011; 6:e19737; PMID:21589864; <http://dx.doi.org/10.1371/journal.pone.0019737>
3. Cunningham C, Wilcockson DC, Boche D, Perry VH. Comparison of inflammatory and acute-phase responses in the brain and peripheral organs of the ME7 model of prion disease. *J Virol* 2005; 79:5174-84; PMID:15795301; <http://dx.doi.org/10.1128/JVI.79.8.5174-5184.2005>
4. Peralta OA, Huckle WR, Eyestone WH. Developmental expression of the cellular prion protein (PrP(C)) in bovine embryos. *Mol Reprod Dev* 2012; 79:488-98; PMID:22674901; <http://dx.doi.org/10.1002/mrd.22057>
5. Ford MJ, Burton LJ, Morris RJ, Hall SM. Selective expression of prion protein in peripheral tissues of the adult mouse. *Neuroscience* 2002; 113:177-92; PMID:12123696; [http://dx.doi.org/10.1016/S0306-4522\(02\)00155-0](http://dx.doi.org/10.1016/S0306-4522(02)00155-0)
6. Raeber AJ, Sailer A, Hegyi I, Klein MA, Rüllicke T, Fischer M, Brandner S, Aguzzi A, Weissmann C. Ectopic expression of prion protein (PrP) in T lymphocytes or hepatocytes of PrP knockout mice is insufficient to sustain prion replication. *Proc Natl Acad Sci U S A* 1999; 96:3987-92; PMID:10097150; <http://dx.doi.org/10.1073/pnas.96.7.3987>

P.216: Multiscale modeling platform for optimization of molecular recognition and translocation of antiprion therapeutic agents

Andriy Kovalenko^{1,2} Neil R Cashman,³ and Nikolay Blinov^{1,2}

¹National Institute for Nanotechnology, Edmonton, AB, Canada; ²Department of Mechanical Engineering, University of Alberta, Edmonton, AB, Canada; ³Department of Medicine, University of British Columbia, Vancouver, BC, Canada

Understanding mechanisms of drugs/antibody recognition and inhibition of neurotoxic aggregates, as well as efficient delivery of therapeutic agents to the central nervous system is important for development of therapy against neurodegenerative diseases.^{1,2} A drug candidate/conformational antibody targeting neurotoxic agents needs to be optimized to cross the blood-brain-barrier. Experimental screening is expensive, and molecular modeling can contribute to initial selection of candidates and optimization of drugs/antibodies functionalization for efficient delivery.³

Modeling of transmembrane translocation in its entirety constitutes a significant challenge, as it spans over a wide range of space and time scales. At present, it is addressed at separate levels of complexity, from the simplistic practical approach of calculating drug partitioning between bulk phases of liquid water and

octanol to estimate the probability of drug translocation across the blood-brain barrier, to novel methods of dissipative particle dynamics simulations, yet with very simplified coarse-grained models, for nanoparticles translocation across biomembranes.

Solvation is a major factor in most biomolecular processes, including protein-ligand recognition and membrane translocation. Such processes often occur on a long-time scale and involve slow conformational changes and exchange of solvent and ligand molecules in biomolecular cavities. Statistical-mechanical, 3D-RISM-KH molecular theory of solvation⁴ accurately describes solvent effects in protein-protein and protein-ligand recognition protocols.^{5,6} In a single formalism, the 3D-RISM-KH method efficiently accounts for electrostatic and non-polar effects, hydrophobicity, structural solvation and desolvation in crowded inter- and intracellular environments.

The new multiscale modeling platform for optimization of molecular recognition and translocation of antiprion therapeutic agents is based on the implementations of the 3D-RISM-Dock protocol in AutoDock suite,^{5,6} 3D-RISM-KH solvent analysis in Molecular Operating Environment package,⁷ and multi-time-step molecular dynamics steered with 3D-RISM-KH effective solvation forces in Amber package.^{8,9} We apply the new platform to study binding modes of antiprion compounds, molecular recognition at the initial stages of oligomerization of A β peptides, structural solvation effects on stability of amyloid fibrils, and optimization of antiprion agents for efficient delivery.

References

1. Frost B, Diamond MI. Prion-like mechanisms in neurodegenerative diseases. *Nat Rev Neurosci* 2010; 11:155-9; PMID:20029438
2. Biran Y, Masters CL, Barnham KJ, Bush AI, Adlard PA. Pharmacotherapeutic targets in Alzheimer's disease. *J Cell Mol Med* 2009; 13:61-86; PMID:19040415; <http://dx.doi.org/10.1111/j.1582-4934.2008.00595.x>
3. Subramanian G, Kitchen DB. Computational models to predict blood-brain barrier permeation and CNS activity. *J Comput Aided Mol Des* 2003; 17:643-64; PMID:15068364; <http://dx.doi.org/10.1023/B:JCAM.0000017372.32162.37>
4. A. Kovalenko, Three-dimensional RISM theory for molecular liquids and solid-liquid interfaces, in: *Molecular Theory of Solvation*, F. Hirata (Ed.) Series: Understanding Chemical Reactivity, vol.24, P. G. Mezey (Ed.), (Kluwer Academic Publishers, Dordrecht, 2003, 360 p.) pp.169-275
5. Nikolić D, Blinov N, Wishart D, Kovalenko A. 3D-RISM-Dock: A New Fragment-Based Drug Design Protocol. *J Chem Theory Comput* 2012; 8:3356-2; <http://dx.doi.org/10.1021/ct300257v>
6. Blinova N, Dorosha L, Wishart D, Kovalenko A. 3D-RISM-KH approach for biomolecular modelling at nanoscale: thermodynamics of fibril formation and beyond. *Mol Simul* 2011; 37:718-8; <http://dx.doi.org/10.1080/08927022.2010.544306>
7. http://www.chemcomp.com/MOE-Structure_Based_Design.htm
8. Luchko T, Gusarov S, Roe DR, Simmerling C, Case DA, Tuszynski J, Kovalenko A. Three-dimensional molecular theory of solvation coupled with molecular dynamics in Amber. *J Chem Theory Comput* 2010; 6:607-24; PMID:20440377; <http://dx.doi.org/10.1021/ct900460m>
9. Omelyan I, Kovalenko A. Multiple time step molecular dynamics in the optimized isokinetic ensemble steered with the molecular theory of solvation: accelerating with advanced extrapolation of effective solvation forces. *J Chem Phys* 2013; 139:244106-23; PMID:24387356; <http://dx.doi.org/10.1063/1.4848716>

P.217: Biological network inferences for protection mechanism against fCJD with E200K

Sol Moe Lee,^{1,†} Myungguen Chung,^{2,†} Kyu Jam Hwang,¹
Young Ran Ju,¹ Jae Wook Hyeon,¹ Jun Sun Park,¹
Sangho Choi,¹ Jeongmin Lee¹, and Su Yeon Kim^{1,*}

¹Division of Zoonoses, Center for Immunology and Pathology and ²Division of Bio-Medical Informatics, Center for Genome Science, National Institute of Health, Korea Center for Disease Control and Prevention; Cheongwon-gun, Republic of Korea

*Correspondence to: Su Yeon Kim; Email: tenksy@korea.kr

[†]These authors contributed equally to this work.

Introduction. The human prion diseases are caused by an abnormal accumulation of misfolded prion protein in the brain. Inherited prion diseases including familial Creutzfeldt-Jakob disease (fCJD) are associated with the mutations of prion protein gene (PRNP). The glutamate to lysine substitution at codon 200 (E200K) in PRNP is the most common pathogenic mutation causing fCJD in the world, but E200K pathogenic mutation alone has been regarded to no sufficient to cause prion diseases and unidentified necessary factors have been proposed to explain penetrance of E200K-dependant fCJD. Here, we analyzed exome differences between fCJD patients with E200K and healthy individuals to find necessary genetic factors and protection mechanisms against CJD with E200K.

Materials and Methods. gDNA samples of 3 CJD patients with E200K, three generation family (n = 12) of one of the CJD patients, and 10 healthy individuals were extracted. Exome sequencing was performed using the HiSeq2000, and CLC Genomics Workbench v5.5 was used to analysis exome sequence data. Fourteen Healthy Korean exome sequences stored in Division of Center for Genome Science in KNIH were used as control during performing variation filtering. Biological network analysis was performed using Cytoscape v3.0, and Pathway studio v9.

Conclusion. Total 19 variations were shown different allele frequencies between cases and controls, and the 19 sites were located on autosome. 18 of the 19 genes harboring the variations haven't been reported prion diseases relation. In our biological network analysis, 4 of the 19 genes harboring the variations were clustered by KEGG pathway criteria such as blood coagulation, aminoacyl-tRNA biosynthesis, and ECM-receptor interaction. Our data implied that a possible mechanism and candidate protecting factor against fCJD with E200K.

P.218: Evaluation of the diagnostic potential of 14-3-3 testing in blood contaminated CSF samples, utilizing sporadic CJD correlated biomarkers (t-Tau, A $\beta_{(1-42)}$)

Angeliki Kokkini,¹ Orgeta Zejneli,¹ Eirini Kanata,¹
Fani Koukouli,² and Theodoros Sklaviadis¹

¹Prion Diseases Research Group, School of Pharmacy, Aristotle University of Thessaloniki; Thessaloniki, Greece; ²Department of Neuroscience, Pasteur Institute; Paris, France

Introduction. Sporadic Creutzfeldt-Jakob disease (sCJD) is the most common form of human prion diseases. Pre-mortem diagnosis is highly desirable. Testing for the presence of 14-3-3 protein in CSF samples of suspected sCJD patients is approved by WHO. This testing is characterized by high sensitivity/specificity and it is used worldwide. However, its usefulness is challenged in blood contaminated CSF samples, resulting in falsely-elevated 14-3-3 outcomes. Recently, it has been suggested that other biomarkers' levels (including t-Tau and A $\beta_{(1-42)}$) in CSF samples may also aid in sCJD diagnosis. Interestingly, the levels of these biomarkers do not seem to be influenced by the presence of blood (within a certain range) in CSF. The aim of the present study was to explore and evaluate the diagnostic accuracy of blood contaminated CSF samples with detectable levels of 14-3-3 by taking into account the levels of t-Tau and A $\beta_{(1-42)}$.

Materials and Methods. We focused on CSF samples from suspected sCJD cases, out of our CSF collection based on blood contamination that was confirmed (Combur test, Roche), prior to western blot for the detection of 14-3-3 protein. Western Blot was performed using a polyclonal sera (anti-14-3-3 β , K19, Sc629, Santa Cruz) and alkaline phosphatase-conjugated as secondary antibody (GAR-AP, Pierce, 31340). Blood-free, 14-3-3 positive and negative samples were used as controls. The intensity of the 14-3-3 protein band was determined by densitometry and the abundance of 14-3-3 in each sample was estimated relative to the positive control. Commercially available ELISA kits (INNOTEST, Innogenetics, Belgium) were used for the determination of t-Tau and A $\beta_{(1-42)}$ levels.

Results. Our preliminary results indicate that t-Tau and A $\beta_{(1-42)}$ could be used to evaluate the significance of 14-3-3 presence in blood contaminated samples. t-Tau levels >1200 pg/ml and A β levels <500 pg/ml increase the possibility of sCJD, while a reversed pattern of these biomarkers is considered indicative of non-sCJD cases. A higher number of samples are being processed in order to confirm this approach.

Conclusions. Our results suggest that in cases where a second Lumbar Puncture is not feasible, blood contaminated samples may be of diagnostic significance when taking into account t-Tau and A $\beta_{(1-42)}$ levels.

Acknowledgment. This research has been supported by the JPND program (1/11/2012-31/10/2015) "Biomarker based diagnosis of rapid progressive dementias-optimization of diagnostic protocols" (DEMTEST).

P.219: Clinical features of Gerstmann-Sträussler-Scheinker syndrome with a P105L mutation and a valine-encoding polymorphic codon 129 in the prion protein gene

Fumiko Furukawa,¹ Nobuo Sanjo,¹ Atsushi Kobayashi,²
Tetsuyuki Kitamoto,² Atsushi Shiraishi,¹ Kinya Ishikawa,¹
Haruhisa Honda,³ Masahito Yamada,⁴
and Hidehiro Mizusawa¹

¹Department of Neurology and Neurological Science; Tokyo Medical and Dental University; Tokyo, Japan; ²Department of Prion Protein Research; Division of CJD Science and Technology; Tohoku University Graduate School of Medicine; Sendai, Japan; ³Department of Neurology; Hatsuishi Hospital; Chiba, Japan; ⁴ Department of Neurology and Neurobiology of Aging; Kanazawa University Graduate School of Medical Science; Kanazawa, Japan

Introduction. Patients having Gerstmann-Sträussler-Scheinker disease (GSS) with the P105L mutation and a valine-encoding polymorphic codon 129 in the prion protein gene (*PRNP*; GSS105) have been reported only from Japan.¹ Here, we present an autopsied GSS105 case with a 21-year clinical course and compare the clinicopathological features of our case with those of other reported cases.

Patient and Methods. A Japanese woman complained of chest and abdomen pain when she was 48, and then her pain migrated to various body parts, but no organic cause of the pain was detected. After hysterectomy, she complained of increasing pain in her entire body. At age 53, she developed cognitive impairment, gait disturbance, and senile gait, and the next year, she showed a confused state and was admitted to a psychiatric hospital under the diagnosis of schizophrenia. When she was 55, a neurologist examined her for the first time and found progressive cognitive impairment, action myoclonus, and pyramidal and extrapyramidal signs. Electroencephalography showed no periodic sharp wave complexes. Magnetic resonance imaging revealed frontal dominant brain atrophy. *PRNP* analysis revealed a P105L mutation and a Met/Val polymorphism at codon 129. At 57, she became bed-ridden and mute. At 59, she could not take any foods orally. At 69, 21 years after the onset, she died. Brain tissue from this patient was homogenized and analyzed using western blotting. Her family history suggested autosomal dominant inheritance. We reviewed surveillance data of her family members and compared them with those of other reported cases.

Results. This patient showed extremely slow clinical progression, and the disease duration was 21 years, which is the longest in all reported Japanese patients with prion disease. A literature review revealed that cognitive impairment (10/10), pyramidal sign (9/10), ataxia (4/9), and postural tremor (6/10) are common clinical features,² but sensory-psychiatric symptoms have not been reported in other patients with GSS105. The variety of clinical symptoms reported for the early stage of GSS105, including ataxia without spasticity, spastic paraparesis, rigidity, postural tremor and cenesthetic hallucination, indicate that the spectrum of GSS105 phenotypes varies among patients² and requires

further clinicopathological elucidation. Western blot analysis of the proteinase K (PK)-resistant fraction from our patient revealed a lower- molecular weight (MW) band at about 6 kDa but no high MW band.

Conclusion. Clinical features of GSS105 vary among patients; this may be associated with the presence or absence of PK-resistant low- and high-MW PrP^{Sc}.

References

1. Yamazaki M, Oyanagi K, Mori O, Kitamura S, Ohya M, Terashi A, Kitamoto T, Katayama Y. Variant Gerstmann-Sträussler syndrome with the P105L prion gene mutation: an unusual case with nigral degeneration and widespread neurofibrillary tangles. *Acta Neuropathol* 1999; 98:506-11; PMID:10541874; <http://dx.doi.org/10.1007/s004010051116>
2. Iwasaki Y, Kizawa M, Hori N, Kitamoto T, Sobue G. A case of Gerstmann-Sträussler-Scheinker syndrome with the P105L prion protein gene mutation presenting with ataxia and extrapyramidal signs without spastic paraparesis. *Clin Neurol Neurosurg* 2009; 111:606-9; PMID:19443103; <http://dx.doi.org/10.1016/j.clineuro.2009.03.008>

P.220: Decoding differences in the replication of sporadic and variant Creutzfeldt-Jakob disease (CJD) in transgenic mice over-expressing human prion protein (TgRM)

Oksana Yakovleva,¹ Paula Saá,¹ Pedro Piccardo,²
Jorge de Castro,¹ Irina Vasilyeva,¹ Anton Cervenak,¹
and Larisa Cervenakova¹

¹American National Red Cross, Holland Laboratory; Rockville, MD, USA; ²LBTSEA/DETDD/OBRR/CBER/FDA; Rockville, MD USA

Keywords: Sporadic Creutzfeldt-Jakob disease, variant Creutzfeldt-Jakob disease, blood infectivity, animal models

In variant CJD (vCJD)-infected individuals, the misfolded prion protein (PrP^{TSE}) accumulates in the lymphoreticular system before the occurrence of clinical disease. Blood of vCJD-infected people contains TSE infectivity, and a few laboratories have identified the presence of PrP^{TSE} in blood of vCJD patients by various methods. Although PrP^{TSE} has not been detected in the blood of patients with sporadic CJD (sCJD), one laboratory has recently reported the presence of infectivity in the plasma of two out of four patients with sCJD. We have failed to transmit the disease in the TgRM model with plasma from symptomatic vCJD- or sCJD-infected mice, while we showed infectivity in plasma of vCJD-infected wild-type mice. To explain these discrepancies, we followed the accumulation of PrP^{TSE} in the brains and spleens of vCJD- and sCJD-infected TgRM mice over time. In addition, we examined the conversion efficiency of both human PrP^{TSE} under various conditions by PMCA.

TgRM mice were injected intracranially with sCJD or vCJD brain homogenates (NIBSC, UK). Mice were euthanized periodically (sCJD at 4, 8, 12, 16, 23, and 24 weeks and vCJD at 6, 12, 26, 39, 52, and 75 weeks) and blood, brains and spleens were removed for biochemical and PMCA studies. Selected brains were examined histologically.

In agreement with previous studies, we showed that vCJD-infected mice develop clinical disease after significantly longer incubation periods than do sCJD-infected mice. However, the accumulation of PrP^{TSE} in the brain of vCJD-infected mice was observed starting approximately half-way through the incubation period, in contrast to sCJD-infected mice in which PrP^{TSE} was first detected by western blotting and immunohistochemistry near the onset of symptomatic disease. PrP^{TSE} in the spleens of vCJD-infected mice was also detected at the preclinical phase, whereas no PrP^{TSE} was identified in the spleens of sCJD-infected mice at any stage of disease. We demonstrated by PMCA that brain-derived PrP^{vCJD} was readily converted in a TgRM substrate and could be serially propagated in vitro, whereas PrP^{sCJD} conversion was very inefficient and gradually disappeared upon repeated rounds of dilution-conversion.

Differences between vCJD and sCJD lymphotropic features observed in humans were reproduced to a certain degree in TgRM mice, but the lack of association in the dynamics of PrP^{TSE} accumulation in brains of sCJD- and vCJD-infected mice with in vitro conversion data remains unexplained. The absence of infectivity in vCJD-infected TgRM plasma in the presence of PrP^{vCJD} accumulation in spleen warrants investigation of infectivity in cellular blood components.

P.221: Novel pomegranate oil nano-emulsions for the prevention and treatment of neurodegenerative diseases: The case of genetic CJD

Ruth Gabizon,¹ Michal Mizrahi,¹ Yael Friedman-Levy,¹ Liraz Larush,² Kati Frid,¹ Orli Binyamin,¹ Dvir Dori,¹ Nina Feinstein,¹
Haim Ovadia,¹ Tamir Ben-Hur,¹ and Shlomo Magdassi²

¹Neurology, Hadassah University Hospital; Jerusalem, Israel; ²Casali Institute of Chemistry, The Hebrew University of Jerusalem; Jerusalem, Israel

Introduction. Prevention of neurodegenerative disease onset and advance by safe compounds that can be administered either to subjects at risk or to those showing early symptoms should be the main target of disease modifying research. Such at risk individuals are carriers of pathogenic mutant PrP forms causing genetic Creutzfeldt-Jacob disease (gCJD).

Materials and methods. Sensitivity to oxidative stress plays a major role in neurodegenerative diseases. Therefore, we treated our TgMHu2ME199K mice, which model for late onset gCJD linked to E200K PrP, with Pomegranate seed oil (PSO), either in its natural form or in a novel nanodroplet formulation (Nano-PSO) which allows PSO components to “escape” the imminent trap of lipid molecules in the liver. PSO comprises large concentrations of a unique conjugated polyunsaturated fatty acid, Punicic acid (PA), considered one of the strongest natural antioxidants. Unsaturated fatty acids can readily pass the BBB and we speculate they can do so more efficiently when their circulation in the blood and immune system is longer, as in Nano-PSO.

Young and asymptomatic mice, as well as older and already sick TgMhu2ME199K mice, were treated for several months either with PSO or with Nano-PSO, followed for disease progression and subsequently their brains tested for prion specific and for general neurodegeneration markers.

Results. Administration of PSO to asymptomatic TgMhu2ME199K mice delayed disease onset to a certain extent, however it was Nano-PSO that delayed both disease onset and progression in a considerably faster mode and lower dose than natural PSO. Only Nano-PSO prevented further advance of disease when administrated to already sick TgMhu2ME199K mice. No aberrant side effect was observed in the time frame and doses used in these experiments. Analysis of brain samples revealed that while PK resistant PrP levels were not affected, brains from PSO and Nano-PSO treated mice exhibited decreased lipid oxidation and neuronal loss, in addition to increased synapthophysin expression and neurogenesis, indicating significant reduction in neurodegeneration markers.

Conclusions. Nano-PSO may be beneficial to subjects suffering from diverse neurodegenerative conditions, including prion diseases. Most important, these compounds are safe enough to be administrated for long periods of time to asymptomatic at risk individuals.

P.222: New studies on the characteristics of VPSPr: PrP^{Sc} and PrP^C

Silvio Notari,¹ Liuting Qing,¹ Qingzhong Kong,¹
Manuel V Camacho,¹ James A Mastrianni,² Wenquan Zou,¹
and Pierluigi Gambetti¹

¹Case Western Reserve University; Cleveland, OH USA; ²University of Chicago Pritzker School of Medicine; Chicago, IL USA

Variably protease-sensitive prionopathy (VPSPr), first described in 2008, is an atypical, apparently sporadic human prion disease which has been observed in at least 53 cases and accounts for approximately 3% of all sporadic prion diseases. However, VPSPr might be more common as it may remain unrecognized owing to its similarity with atypical dementias. Although several aspects of this disorder have been characterized, transmissibility of VPSPr and the molecular underpinning of its dissimilarity with other sporadic prion diseases remain largely unknown.

We now report on the in vitro propagation of VPSPr-associated PrP^{Sc}, using protein misfolding cyclic amplification (PMCA). As VPSPr affects all three 129 prion protein (PrP) genotypes—VV, MV and MM—we tested the importance of residue 129 seed-substrate pairings. Using human PrP^C from transgenic (Tg) mice with the same expression levels, the most efficient amplification was obtained seeding VPSPr-MM to PrP^C-M and VPSPr-VV to PrP^C-V. VPSPr-MV amplified as VPSPr-MM. The efficiency of VPSPr amplification by PMCA, in contrast to more common sCJD subtypes, decreased after the 1st round. These results are

consistent with those obtained by bioassay on Tg mice expressing human PrP^C (Gambetti et al., unpublished data). They indicate that VPSPr PrP^{Sc} can convert human PrP^C but in a way that is less efficient than, and perhaps different from, that of sporadic Creutzfeldt-Jakob disease (sCJD) PrP^{Sc}.

With regard to the molecular basis of VPSPr, we recently observed that approximately half of the soluble, PK-sensitive “PrP^C” present in VPSPr (but not in sCJD and sFI, the other two forms of sporadic human prion disease) brain homogenate is truncated at the N-terminus. Based on epitope mapping and electrophoretic mobility, we estimate that this truncation spans residues 23–33 (hereafter referred to as $\Delta 23-33$), even though no mutation has been found in the PrP gene open reading frame. Cleavage of the GPI-anchor with hydrofluoric acid and immunoreactivity with antibodies to the C-terminus of PrP clearly show that PrP ^{$\Delta 23-33$} , like normal PrP, has a complete C-terminus that includes the anchor. N-terminal sequencing is ongoing. In several studies the polybasic 23-28 region of PrP has been implicated in endocytic trafficking, membrane interaction and binding to glycosaminoglycans, suggesting a role of this region in PrP^{Sc} formation and propagation. Whether and how this peculiar N-terminal PrP truncation plays a role on VPSPr pathogenesis, remains to be determined.

Acknowledgments. Supported by NIH Awards AG14359 and NS062787. CDC Award UR8/CCU515004, Charles S. Britton Fund.

P.223: Regional distribution of anchorless prion protein, PrP226*, in the human brain

Anja Lukan,¹ Maja Černilec,¹ Tanja Vranac,¹ Mara Popović,²
and Vladka Čurin Šerbec¹

¹Department for the Production of Diagnostic Reagents and Research; Blood Transfusion Centre of Slovenia; Ljubljana, Slovenia; ²Institut for Pathology; Medical Faculty; University of Ljubljana; Ljubljana, Slovenia

Truncated forms of prion protein (PrP) can be found in brains of patients with some types of transmissible spongiform encephalopathies (TSE). N-terminal as well as C-terminal truncated forms of prion protein have been described.^{1–3} Notari et al. found a fragment of prion protein which lacks a few amino acids at the very end of the C terminus together with the GPI anchor in sporadic and variant Creutzfeldt-Jakob disease (CJD) patients, but the exact point of truncation has not been determined in their study.¹ The importance of anchorless PrP molecules in TSE pathogenesis has been further demonstrated by different studies.^{4–6} Our group reported on the discovery of an anchorless PrP fragment in the brain of sCJD patients, truncated at Tyr226, named PrP226*, by mAb V5B2 which was developed by our group and specifically recognizes PrP226*.^{7,8}

In the present study regional distribution of PrP226* in five different regions of numerous human brain was assessed using a sandwich ELISA based on mAb V5B2, designed especially for

this purpose.⁹ It was predicted that PrP226* accumulates in diseased brain in aggregates, from which it can be released using chaotropic salts.

We found that PrP226* indeed accumulates in aggregates during the disease, but is also present naturally in brain in minute amounts. With our ELISA test we were able to follow the distribution of this fragment. PrP226* is most likely to accumulate in cerebellum, followed by cortical regions, but very rarely in spinal cord or olfactory bulb.¹⁰ Comparison of the results for PrP226* and PrP^{Sc} revealed that the distribution of both proteins correlates well, and strongly indicates that PrP226* is part of PrP^{Sc} aggregates (Lukan and Černilec, et al. under revision).

References

1. Notari S, Strammiello R, Capellari S, Giese A, Cescatti M, Grassi J, Ghetti B, Langeveld JP, Zou WQ, Gambetti P, et al. Characterization of truncated forms of abnormal prion protein in Creutzfeldt-Jakob disease. *J Biol Chem* 2008; 283:30557-65; PMID:18753138; <http://dx.doi.org/10.1074/jbc.M801877200>
2. Zanusso G, Farinazzo A, Prelli F, Fiorini M, Gelati M, Ferrari S, Righetti PG, Rizzuto N, Frangione B, Monaco S. Identification of distinct N-terminal truncated forms of prion protein in different Creutzfeldt-Jakob disease subtypes. *J Biol Chem* 2004; 279:38936-42; PMID:15247220; <http://dx.doi.org/10.1074/jbc.M405468200>
3. Zou WQ, Capellari S, Parchi P, Sy MS, Gambetti P, Chen SG. Identification of novel proteinase K-resistant C-terminal fragments of PrP in Creutzfeldt-Jakob disease. *J Biol Chem* 2003; 278:40429-36; PMID:12917418; <http://dx.doi.org/10.1074/jbc.M308550200>
4. Chesebro B, Trifilo M, Race R, Meade-White K, Teng C, LaCasse R, Raymond L, Favara C, Baron G, Priola S, et al. Anchorless prion protein results in infectious amyloid disease without clinical scrapie. *Science* 2005; 308:1435-9; PMID:15933194
5. Chesebro B, Race B, Meade-White K, Lacasse R, Race R, Klingeborn M, Striebel J, Dorward D, McGovern G, Jeffrey M. Fatal transmissible amyloid encephalopathy: a new type of prion disease associated with lack of prion protein membrane anchoring. *PLoS Pathog* 2010; 6:e1000800; PMID:20221436; <http://dx.doi.org/10.1371/journal.ppat.1000800>
6. Stöhr J, Watts JC, Legname G, Oehler A, Lemus A, Nguyen HO, Sussman J, Wille H, DeArmond SJ, Prusiner SB, et al. Spontaneous generation of anchorless prions in transgenic mice. *Proc Natl Acad Sci U S A* 2011; 108:21223-8; PMID:22160704; <http://dx.doi.org/10.1073/pnas.1117827108>
7. Kosmač M, Koren S, Giachin G, Stoilova T, Gennaro R, Legname G, Serbec VČ. Epitope mapping of a PrP(Sc)-specific monoclonal antibody: identification of a novel C-terminally truncated prion fragment. *Mol Immunol* 2011; 48:746-50; PMID:21176851; <http://dx.doi.org/10.1016/j.molimm.2010.11.012>
8. Curin Serbec V, Bresjanac M, Popovic M, Pretnar Hartman K, Galvani V, Ruprecht R, Černilec M, Vranac T, Hafner I, Jerala R. Monoclonal antibody against a peptide of human prion protein discriminates between Creutzfeldt-Jacob's disease-affected and normal brain tissue. *J Biol Chem* 2004; 279:3694-8; PMID:14593100; <http://dx.doi.org/10.1074/jbc.M310868200>
9. Dvorakova E, Vranac T, Janouskova O, Černilec M, Koren S, Lukan A, Nováková J, Matej R, Holada K, Čurin Šerbec V. Detection of the GPI-anchorless prion protein fragment PrP226* in human brain. *BMC Neurol* 2013; 13:126; PMID:24063733; <http://dx.doi.org/10.1186/1471-2377-13-126>

P.224: Evaluation of assays intended for the diagnosis of variant CJD

Jillian K Cooper, Kaetan Ladhani, and Philip D Minor

CJD Research and Resource Centre, National Institute for Biological Standards and Control (Medicines and Healthcare Products Regulatory Agency); South Mimms, Hertfordshire, UK

Keywords: variant CJD, diagnostic test, evaluation

Tests intended for the diagnosis of potentially life threatening disorders are listed in Annex IIA of the EU directive and have

associated minimum requirements outlined in a common technical specification. Variant CJD diagnostic tests were added to Annex IIA in 2011.

Typically tests are evaluated for sensitivity using a large number of clinically relevant samples, for vCJD this is not possible due to the small number of clinical cases and the very limited number of relevant samples. To help ensure fair and appropriate access to the small number of rare samples held at the UK CJD Resource Centre its oversight committee have developed a process to assess sensitivity and specificity (http://www.nibsc.org/Spotlight/CJD_Resource_Centre/CJD_Tests.aspx).

This process involves analysis of 1. vCJD tissue homogenates spiked into the relevant blood component (to determine analytical sensitivity) 2. Blood components from experimentally or naturally infected animals (diagnostic sensitivity) 3. Blood components from normal donor samples (for diagnostic specificity).

Each stage of the evaluation process is reviewed by the oversight committee and if minimum requirements are met samples from vCJD clinical cases are provided for testing.

Two test developers have completed the evaluation process and tested blood components from clinical cases of vCJD held at the UK CJD Resource Centre.¹

References

1. Cooper JK, Ladhani K, Minor P. Comparison of candidate vCJD in vitro diagnostic assays using identical sample sets. *Vox Sang* 2012; 102:100-9; PMID:22126309; <http://dx.doi.org/10.1111/j.1423-0410.2011.01525.x>

P.225: A newly developed assay for the presymptomatic detection of prions in blood

Daisy Bougard,¹ Maxime Belondrade,¹ Christiane Segarra,¹ Vincent Béringue,² and Joliette Coste¹

¹Laboratoire TransDiag, Sécurité Transfusionnelle et Innovation Diagnostique, Etablissement Français du Sang Pyrénées Méditerranée; Montpellier, France; ²INRA UR892 Virologie Immunologie Moléculaires; Jouy-en-Josas, France

Introduction. Prion diseases or Transmissible Spongiform Encephalopathies (TSEs) are neurodegenerative diseases including variant Creutzfeldt-Jakob disease (vCJD) in humans. The central event of these diseases would be the conformational change of a normal cellular protein PrP^C into an infectious form PrP^{TSE}. It is now evident that TSEs are transmissible by blood transfusion and this has raised concerns that a reservoir of infectious asymptomatic people could exist in the blood donor population. A recent prevalence study based on stored lymphoreticular samples analyses led to an estimation of 1 in 2000 persons being potentially infected by vCJD prions in the United Kingdom. Until now, no screening test could detect the infectious agent in human blood before the onset of clinical signs of disease.

The objective of this study is to develop a sensitive and specific test that would enable the detection of PrP^{TSE} in the blood during the presymptomatic phase of TSE.

Materials and Methods. The detection assay comprises three major steps: (1) a ligand-coated bead pre-analytical step in order

to concentrate PrP^{TSE} from the different blood components and to remove inhibitory factors which can interfere with the amplification; (2) a PrP^{TSE} amplification by serial PMCA using transgenic mouse brain homogenate as substrate and (3) a specific detection of the amplified PrP^{TSE} by immuno-blotting after partial proteinase K digestion. The sample volume has been optimized for 500 µL of plasma and for 25 to 50 µL of buffy-coat. Whole blood samples from infected sheep collected during preclinical and clinical phases of scrapie were processed in buffy-coat, white blood cells (WBC) and plasma. Blood from humanised transgenic mice infected by vCJD was also tested in relation with the duration post inoculation.

Results. PMCA assay allowed detection of PrP^{TSE} in: (1) the WBC of four sheep at the acute phase of scrapie with a 100% sensitivity and specificity, (2) in the plasma and buffy coat collected in the asymptomatic phase of the disease (3) in the blood of vCJD-infected transgenic mice before the occurrence of clinical signs.

Conclusions. The expected level of sensitivity for the detection of prion in the blood was reached. This assay is currently evaluated as a confirmatory detection test for the presence of the vCJD agent in human blood. The next step will be to perform prevalence studies by analysing panels of at-risk populations.

P.226: Neurodegeneration and neurogenesis in the gastrointestinal tract of prion infected mice

Laura Ellett,¹ Amanda Quattrocchi,¹ Richard Schregle,¹
Ewan Chan,¹ Gene Venables,² John B Furness,²
and Victoria A Lawson¹

¹Department of Pathology; The University of Melbourne, Parkville, VIC Australia;

²Department of Anatomy and Neuroscience; The University of Melbourne, Parkville, VIC Australia

The presence of disease associated PrP^{Sc} in the gastrointestinal tract of patients affected by prion diseases represents a source of disease transmission through surgical procedures and may contribute to symptoms affecting their quality of life.

Following intracerebral inoculation of wildtype mice with prions, PrP^{Sc} was detected in the gastrointestinal tract during symptomatic disease (20 weeks post infection). PrP^{Sc} accumulation in the ileum was associated with high levels of infectivity, caspase activation, loss of neuronal subpopulations in the myenteric plexus and the loss of enteric glial cell integrity.^{1, 2} In the current study a time course analysis indicated that PrP^{Sc} accumulation was detected in the ileum within 3 weeks of intracerebral inoculation, with highest levels detected 6-10 weeks post-inoculation, at which time mice demonstrated no neurological symptoms of disease. There was a quantitative decrease in PrP^{Sc} load 13 weeks post infection suggestive of a shedding of PrP^{Sc} from the gastrointestinal tract and a potential source of environmental contamination.

A significant loss of neurofilament (medium) immunoreactive neurons, reflective of intrinsic sensory neurons, was detected 10

weeks post inoculation and coincident with high PrP^{Sc} loads. This was followed by the apparent recovery of this neuronal population between weeks 13 and 17 post-inoculation and coincident with reduced PrP^{Sc} loads. These results suggest that the accumulation of PrP^{Sc} may directly contribute to the loss of this neuronal population and that a reduction in PrP^{Sc} burden enabled its regeneration.

We had previously observed the qualitative loss of neuronal nitric oxide synthase (nNOS) immunoreactive neurons, a marker of inhibitory motor neurons, in conjunction with regions of distorted glial fibrillary acidic protein (GFAP) immunoreactivity, indicative of enteric glial cell derangement at the terminal stages of disease. We now report a significant decrease in nNOS immunoreactive neurons between 10 and 17-week post inoculation and evidence of enteric glial cell derangement as early as 6 weeks post inoculation. These results support the view that glial cell dysfunction in the gastrointestinal tract of prion affected animals precedes and contributes to the loss of nNOS immunoreactive neurons.

This study has identified PrP^{Sc} accumulation and glial cell dysfunction as mediators of prion induced neurodegeneration of specific neuronal subpopulations within the gastrointestinal tract and the potential for regeneration of neuronal populations following a reduction in PrP^{Sc} load. The effect of neuronal loss on gastrointestinal dysfunction will be presented.

References

1. Lawson VA, Furness JB, Klemm HM, Pontell L, Chan E, Hill AF, Chiocchetti R. The brain to gut pathway: a possible route of prion transmission. *Gut* 2010; 59:1643-51; PMID:21071583; <http://dx.doi.org/10.1136/gut.2010.222620>
2. Drew SC, Haigh CL, Klemm HM, Masters CL, Collins SJ, Barnham KJ, Lawson VA. Optical imaging detects apoptosis in the brain and peripheral organs of prion-infected mice. *J Neuropathol Exp Neurol*. 2011; 70:143-50; PMID: 21343883; <http://dx.doi.org/10.1097/NEN.0b013e3182084a8c>.

P.227: Selective binding of high molecular mass assemblies of amyloid β-peptide to prion protein in patients with Alzheimer's disease

Frank Dohler,¹ Diego Sepulveda-Falla,¹ Susanne Krasemann,¹
Hermann Altmeyen,¹ Hartmut Schlueter,² Inga Zerr,³
Jakob Matschke,¹ and Markus Glatzel¹

¹Institute of Neuropathology, University Medical Centre Hamburg-Eppendorf; Hamburg, Germany, ²Mass Spectrometric Proteomics Group Institute of Clinical Chemistry, University Medical Centre Hamburg-Eppendorf; Hamburg, Germany;

³Department of Neurology, National TSE Reference Centre, Georg-August University Goettingen; Goettingen, Germany

In Alzheimer's disease the generation of oligomeric species of amyloid β-peptide is causal to disease initiation and progression. Oligomeric species of amyloid β-peptide bind to the N-terminus of plasma membrane-bound cellular prion protein (PrP^C). This binding may be associated to synaptic degeneration. Composition of bound amyloid β-peptide oligomers, binding domains within PrP^C and modifiers of this binding have mostly been studied in cell culture or murine models of Alzheimer's disease. Our

understanding on the PrP^C amyloid β -interaction in patients with Alzheimer's disease is limited regarding to occurrence of binding, binding domains within PrP^C, and sizes of bound oligomeric species of amyloid β -peptide.

Here we employed the combination of a novel Amyloid- β -PrP^C interaction assay (APIA), co-immunoprecipitation and size exclusion chromatography to study occurrence of PrP^C amyloid β -peptide binding on neuropathologically characterized Alzheimer's disease and control brains. We investigated binding-specificity, binding domains of PrP^C and sizes of bound oligomeric species of amyloid β -peptide.

Amyloid β -peptide binding to PrP^C always occurred in Alzheimer's disease brains and was never detected in non-demented controls. Expression level of PrP^C or known genetic modifiers of Alzheimer's disease, such as the PrP^C codon 129 polymorphism, do not act as modifiers of this interaction. Binding domains are found in the N-terminus of PrP^C. Sizes of oligomeric species of amyloid β -peptide differ between synthetic amyloid β -peptide and Alzheimer's disease brain-derived amyloid β -peptide where larger protein assemblies containing amyloid β -peptide efficiently bind to PrP^C.¹

References

1. Dohler F, Sepulveda-Falla D, Krasemann S, Altmepfen H, Schlüter H, Hildebrand D, Zerr I, Matschke J, Glatzel M. High molecular mass assemblies of amyloid- β oligomers bind prion protein in patients with Alzheimer's disease. *Brain* 2014; 137:873-86; PMID:24519981; <http://dx.doi.org/10.1093/brain/awt375>

P.228: Dose-response for vCJD transmitted through blood transfusion

Hong Yang,¹ Yin Huang,¹ Steven A Anderson,¹ Luisa Gregori,² and David M Asher²

¹Office of Biostatistics and Epidemiology; Center for Biologics Evaluation and Research; US Food and Drug Administration; Rockville, MD USA; ²Office of Blood Evaluation and Research; Center for Biologics Evaluation and Research; US Food and Drug Administration; Rockville, MD USA

Keywords: dose-response, vCJD, transfusion-transmission

What is the likelihood that a unit of blood donated by an individual infected with variant Creutzfeldt-Jakob disease (vCJD) will infect a recipient? This information is critical to estimate the risk of transfusion-transmitted vCJD (TTvCJD). Previous estimates of possible vCJD infectivity titers in human blood were derived from the infectivity titers of rodent blood experimentally infected with model transmissible spongiform encephalopathy (TSE) agents, assayed by intracerebral inoculations into rodents of the same species. Those estimates relied on an assumption that titers of the agent in the blood of an infected person during the incubation period of vCJD is likely to be similar to that of an infected rodent.

We have conducted two analyses to evaluate the probable dose-response relationship for TTvCJD in humans. In the first

study¹ we analyzed UK data for recipients of vCJD-implicated (donor later developed vCJD) blood components. We back-calculated the potential number of infected recipients who were asymptomatic based on the number of reported TTvCJD cases, the incubation periods of vCJD and survival rate of blood recipients. We estimated the probability of vCJD infection in a recipient transfused with a unit of infectious blood based on total number of recipients and estimate of the number of recipients who have been infected. In a more recent analysis, we developed a dose-response model using published data from a large study in which non-human primates were inoculated intracerebrally with suspensions of brain tissue from CJD patients (Brown et al. *Ann Neurol* 1994;35:513). The dose-response model described the relationship between the amount of brain tissue injected and the probability of infection in primates. We then adjusted model parameters to account for differences in infectivity between blood and brain tissue and efficiency of vCJD transmission between intravenous and intracerebral routes. The resulting dose-response model described the relationship between the amount of transfused blood and the probability of infecting a recipient.

The first analysis estimated the mean probability of vCJD infection from transfusion of a unit of non-leukoreduced red blood cells from a donor in late incubation period of vCJD to be 75% (90% CI 56-96%). The dose-response model developed in the recent analysis indicated a mean probability of infection from transfusion of a unit of infected whole blood to be 76% (90% CI 48-90%). Results of the two analyses are consistent. The update of dose-response will improve accuracy of risk assessment for TTvCJD.

References

1. Gregori L, Yang H, Anderson S. Estimation of variant Creutzfeldt-Jakob disease infectivity titers in human blood. *Transfusion* 2011; 51:2596-602; PMID:21645006; <http://dx.doi.org/10.1111/j.1537-2995.2011.03199.x>

P.229: Transgenic mice expressing bank vole prion protein spontaneously produce transmissible prions indistinguishable from an inherited human prion disease

Romolo Nonno,¹ Laura Pirisinu,¹ Michele A Di Bari,¹ Natalia Fernández-Borges,² Manuel A Sánchez-Martín,³ Enric Vidal,⁴ Claudia D'Agostino,¹ Stefano Marcon,¹ Geraldina Riccardi,¹ Pierluigi Gambetti,⁵ Joaquín Castilla,² and Umberto Agrimi¹

Department of Veterinary Public Health and Food Safety, Istituto Superiore di Sanità; Rome, Italy; ²CIC bioGUNE and IKERBASQUE, Basque Foundation for Science; Derio and Bilbao, Bizkaia, Spain; ³Unidad de Generación de OMGs, S.E.A. Department of Medicine, University of Salamanca; Salamanca, Spain; ⁴Centre de Recerca en Sanitat Animal (CRESA), UAB-IRTA, Campus de la Universitat Autònoma de Barcelona; Bellaterra, Barcelona, Spain; ⁵Case Western Reserve University; Cleveland, OH USA

Bank voles are strikingly susceptible to human and animal prions,¹ and it has been shown that transgenic mice expressing

a natural polymorphic vole PrP variant, coding for isoleucine at codon 109 (BvPrP109I), spontaneously develop a prion disease associated with comparatively protease-sensitive PrP.²

We generated a new line of transgenic mice, named TgL1, expressing BvPrP109I and developing a spontaneous neurological disease. We studied the clinico-pathological characteristics of this disease, along with its biological features by bioassay in homologous wild type bank voles (Bv109I).

All TgL1 mice showed spontaneous neurological illness characterized by ataxia, proprioceptive deficits, kyphosis and hind limb paresis, and invariably progressed to terminal disease with a survival of 182±36 days. Neuropathological analysis showed spongiform degeneration, gliosis, neuronal loss and PrP^{Sc} deposition in all spontaneously ill mice. The severity and distribution of these changes were variable, with cerebral cortex, striatum and thalamus being the most frequently affected areas, while cerebellum and hippocampus were either spared or heavily affected. White and grey matter granular and punctuate PrP^{Sc} deposition was accompanied in some mice by plaques, including multicentric plaques similar to those found in Gerstmann-Sträussler-Scheinker disease (GSS). PK-resistant PrP^{Sc} was detected in all mice, with a uniform GSS-like pattern characterized by a 7-8 kDa internal PrP fragment.

Brain homogenates from 5 spontaneously ill TgL1 mice, representing the observed clinico-pathological variability, were selected for bioassay. All inocula transmitted to voles with survival time of ~100 days and produced a uniform disease phenotype, irrespective of the pathological variations observed in the donor brain, characterized by a GSS-like PrP^{Sc} signature. By end-point dilution, we found that TgL1 mice spontaneously accumulate up to 10^{8.5} ID₅₀/g.

Prompted by the similarities with GSS, we inoculated GSS cases in Bv109I and found that cases with A117V mutation transmitted to Bv109I in ~100 days, without transmission barrier and with a disease phenotype indistinguishable from that observed in Bv109I inoculated with spontaneously ill TgL1 mice.

In conclusion, we show that TgL1 mice recapitulate the hallmarks of prion diseases, including PK-resistant PrP^{Sc} and high prion titer, mimic the clinico-pathological variability observed in some inherited human prion disease and spontaneously produce prions indistinguishable from those found in GSS A117V cases. Therefore, TgL1 represents a valuable animal model for studying the mechanisms of spontaneous formation of prions and the causes of the phenotypic variability observed in inherited prion disorders, as well as to test drugs able to modify/prevent the progression of these disorders.

References

1. Nonno R, Di Bari MA, Cardone F, Vaccari G, Fazzi P, Dell'Omo G, Cartoni C, Ingrosso L, Boyle A, Galeno R, et al. Efficient transmission and characterization of Creutzfeldt-Jakob disease strains in bank voles. *PLoS Pathog* 2006; 2:e12; PMID:16518470; <http://dx.doi.org/10.1371/journal.ppat.0020012>
2. Watts JC, Giles K, Stöhr J, Oehler A, Bhardwaj S, Grillo SK, Patel S, DeArmond SJ, Prusiner SB. Spontaneous generation of rapidly transmissible prions in transgenic mice expressing wild-type bank vole prion protein. *Proc Natl Acad Sci U S A* 2012; 109:3498-503; PMID:22331873; <http://dx.doi.org/10.1073/pnas.1121556109>

P.230: Algorithm for the neuropathologic diagnosis of prion diseases

Wiebke M Wemheuer, Arne Wrede, Insa Damman, and Walter J Schulz-Schaeffer

University medical centre Goettingen (UMG); Goettingen, Germany

Background/Introduction. Prion diseases or transmissible spongiform encephalopathies are always fatal and potentially infectious to those who handle the tissue. The diagnosis has consequences for hospital hygiene and the reuse of instruments. Therefore, in brain biopsies and autopsy tissues taken from patients with rapidly progressive dementia, the first differential diagnoses to be ruled out are prion diseases. For safe diagnostic processing, a fast, highly sensitive and specific analysis for prion aggregates is utterly necessary.

Material and Methods. Our study aims to validate four methods for diagnosing prion diseases. We examined 16 brain biopsies and brain sections from 65 autopsies with Western blot, paraffin-embedded tissue (PET) blot, immunohistochemistry and the recently described membrane adsorption assay (MAA) for their suitability to detect pathological prion protein in the brain in general and in the brain biopsy site in particular.

Results. The PET blot method provides the highest sensitivity in prion detection (biopsies: 100%; autopsy sections: 96.3%), closely followed by the MAA (biopsies: 100%; autopsy sections: 96%) and western blot analysis (biopsies: 100%; autopsy sections: 94.7%). Conventional immunohistochemistry is the least sensitive method (biopsies: 50%; autopsy sections: 78.8%) and also the one with a false positive biopsy result.

Conclusions. We propose a diagnostic standard that uses the MAA as a first step to detect or exclude a prion disease, followed by PET blot, Western blotting for prion typing and immunohistochemistry for differential diagnoses. With this procedure and the availability of unfixed tissue, all 16 examined biopsies were diagnostic. For the benefit of patients and staff we suggest including the MAA and rethinking the role of immunohistochemistry as the “gold standard” of prion disease diagnostics.

P.231: RT-QuIC sensitivity governed by substrate composition, conformation and concentration

Keding Cheng, Angela Sloan, Robert Vendramelli, Kristen M Avery, Anne Peterson, Lise Lamoureux, Sharon Simon, Debra Godal, and J David Knox

National Microbiology Laboratory, Public Health Agency of Canada; Winnipeg, MB Canada

Keywords: CJD, RT-QuIC, rPrP, CSF, diagnoses, ante-mortem

Introduction. Current ante-mortem tests for Creutzfeldt Jakob Disease (CJD) are based upon the detection of surrogate

markers of neuronal damage in cerebral spinal fluid (CSF) and therefore not specific for CJD. For this reason there is considerable interest in a recently developed procedure called real-time quaking-induced conversion (RT-QuIC). RT-QuIC exploits the ability of minute amounts of disease associated prions (PrP^d) in test samples to seed the conformational conversion of soluble recombinant prion protein (rPrP^c) into misfolded proteins that interact with one another to form amyloid aggregates. The successful detection of prions in the 263K hamster scrapie model and in human sporadic CJD (sCJD) patient samples have demonstrated the potential of RT-QuIC to provide a test for prion disease diagnosis based upon the presence of the etiologic agent.¹⁻⁴ To date there is no agreed upon standard procedure for optimal analytical performance.

Previously, using our in-house generated full length hamster prion protein (rHa-PrP^c) as substrate, we developed conditions where reactions seeded with sCJD brain homogenate dilutions up to 10⁻⁸ were reliably detected by RT-QuIC. These conditions were not sufficiently sensitive to detect PrP^d in the CSF of patients with pathologically confirmed CJD. In order to further optimise the method, two different RT-QuIC substrates and more permissive conversion conditions were tested.

Method. The substrates were recombinant full length hamster prion protein (rHa-Prp) and sheep-hamster chimeric prion protein (rSH-PrP) purified as previously described.⁵ The control seeds were commercial brain homogenates (BH) confirmed to be infected with sCJD and non-CJD BH. When diluted in series, the BHs served as positive and negative controls in RT-QuIC reactions. The test samples were CSF obtained from patients with confirmed CJD.

Preliminary Data. Using the same reaction parameters, the rSH-PrP substrate showed higher sensitivity than rHa-PrP, but was more prone to spontaneous aggregation after long incubation periods. Controlled chemical perturbation of rPrP conformation was found to promote conversion.

Conclusion. In order to achieve the level of sensitivity required to reliably detect the vastly lower amounts of PrP^d in CSF, we were obliged to radically revise our views of what constituted the optimal conditions for RT-QuIC.

References

1. Wilham JM, Orrú CD, Bessen RA, Atarashi R, Sano K, Race B, Meade-White KD, Taubner LM, Timmes A, Caughey B. Rapid end-point quantitation of prion seeding activity with sensitivity comparable to bioassays. *PLoS Pathog* 2010; 6:e1001217; PMID:21152012; <http://dx.doi.org/10.1371/journal.ppat.1001217>
2. Peden AH, McGuire LI, Appleford NE, Mallinson G, Wilham JM, Orrú CD, Caughey B, Ironside JW, Knight RS, Will RG, et al. Sensitive and specific detection of sporadic Creutzfeldt-Jakob disease brain prion protein using real-time quaking-induced conversion. *J Gen Virol* 2012; 93:438-49; PMID:22031526; <http://dx.doi.org/10.1099/vir.0.033365-0>
3. Atarashi R, Satoh K, Sano K, Fuse T, Yamaguchi N, Ishibashi D, Matsubara T, Nakagaki T, Yamanaka H, Shirabe S, et al. Ultrasensitive human prion detection in cerebrospinal fluid by real-time quaking-induced conversion. *Nat Med* 2011; 17:175-8; PMID:21278748; <http://dx.doi.org/10.1038/nm.2294>
4. McGuire LI, Peden AH, Orrú CD, Wilham JM, Appleford NE, Mallinson G, Andrews M, Head MW, Caughey B, Will RG, et al. Real time quaking-induced conversion analysis of cerebrospinal fluid in sporadic Creutzfeldt-Jakob disease. *Ann Neurol* 2012; 72:278-85; PMID:22926858; <http://dx.doi.org/10.1002/ana.23589>
5. Cheng K, Sloan A, Avery KM, Coulthart M, Carpenter M, Knox JD. Exploring physical and chemical factors influencing the properties of recombinant prion protein and the real-time quaking-induced conversion (RT-QuIC) assay. *PLoS One* 2014; 9:e84812; PMID:24404191; <http://dx.doi.org/10.1371/journal.pone.0084812>

P.232: Association of prion protein geno- and PrP^{Sc} type with PrP^C charge isoform profiles in cerebrospinal fluid of humans with sporadic or familial prion diseases

Matthias Schmitz, Katharina Lüllmann, Saima Zafar, and Inga Zerr

Department of Neurology, Clinical Dementia Center and DZNE Georg-August University; Göttingen, Germany

Keywords: Biomarker, Creutzfeldt-Jakob disease, PrP^C charge isoforms, truncated PrP^C forms

Introduction. The presence of cellular prion protein (PrP^C) in human cerebrospinal fluid (CSF) had already been demonstrated by Tagliavini and colleagues.¹ In this study we examined if post-translational modifications of PrP^C in the CSF of prion disease patients are associated with methionine (M) / valine (V) polymorphism at codon 129 of the prion protein gene (*PRNP*), PrP^{Sc} type in sporadic Creutzfeldt-Jakob disease (sCJD), or *PRNP* mutations in familial Creutzfeldt-Jakob disease (fCJD/E200K) and fatal familial insomnia (FFI).

Materials and Methods. We performed comparative two-dimensional immunoblotting of PrP^C charge isoforms in CSF samples from a cohort of diseased and control patients. The cohort consisted of sCJD, fCJD (E200K) and FFI (D178N) patients, as well as control donors without prion disease.

Results and Conclusion: We quantified the 12 most abundantly expressed PrP^C isoforms (IF) in the analysed CSF. The expression of only one isoform (IF12) was markedly reduced in sCJD (VV) patients, in sCJD with PrP^{Sc} type 2 and in FFI patients. Interestingly, truncated PrP^C fragments, detected in sCJD and control CSF, exhibited no significant differences.

Conclusion. In conclusion, the obtained data indicated an association between PrP^C charge isoform pattern and *PRNP* codon 129 genotype, PrP^{Sc} type and the type of human prion disease. Because the majority of the sCJD (VV) patients and FFI patients (MM and MV) express PrP^{Sc} type 2, we suggest that IF12 is associated with PrP^{Sc} type 2. If our observations result from a modulation of the physiological PrP^C metabolism remains to be established.

References

1. Tagliavini F, Prelli F, Porro M, Salmona M, Bugiani O, Frangione B. A soluble form of prion protein in human cerebrospinal fluid: implications for prion-related encephalopathies. *Biochem Biophys Res Commun* 1992; 184:1398-404; PMID:1375461; [http://dx.doi.org/10.1016/S0006-291X\(05\)80038-5](http://dx.doi.org/10.1016/S0006-291X(05)80038-5)

P.233: Prion-like aggregation of p53 in breast cancer and glioblastoma: New targets for antitumoral drugs

Jerson L Silva,¹ Danielly C F da Costa,¹ Flavia R S Lima,² Murilo M Pedrote,¹ Guilherme G A P Oliveira,¹ and Luciane P Rangel¹

¹Instituto de Bioquímica Médica Leopoldo de Meis, Universidade Federal do Rio de Janeiro; Rio de Janeiro, RJ, Brazil; ²Instituto de Ciências Biomédicas, Universidade Federal do Rio de Janeiro; Rio de Janeiro, RJ, Brazil

p53 is a master regulatory protein that participates in cellular processes such as apoptosis, DNA repair and cell cycle control. p53 is a tetrameric protein in which each subunit consists of 393 amino acid residues. The function of this tumor suppressor protein is lost in more than 50% of human cancers.¹ Recent studies have suggested that the formation of prion-like aggregates of mutant p53 is associated with loss-of-function, dominant-negative and gain-of-function (GoF) effects.²⁻⁸ Studies from our group have shown that p53 aggregation in a mixture of oligomers and fibrils that sequesters the native protein into an inactive conformation, typical of a prionoid behavior.² These aggregates are present in tissue biopsies of breast cancer especially in more aggressive ones.^{2,9} More recently, we have evaluated whether p53 aggregates may be also present in human glioblastoma cells and participate in the tumorigenic process. Our data shows great colocalization between p53 and oligomers as well as amyloid fibers in glioblastoma cells. The prionoid properties of p53 aggregates are considered potential targets for drug development. The prion-like properties of nucleation, templating, multiplication and spread have been already considered potential new targets for the development of neurodegenerative disease therapies.^{10, 11} We have evaluated whether PRIMA-1, a classical drug described to stabilize mutant p53 structure and function, exerts its effect on aggregated mutant p53. Also, 2-methylene-3-quinuclidinone hydrate (MQ) has been shown to inhibit WT and mutant recombinant p53 central core domain (p53C) aggregation at 37°C. The WT form has been protected in a lower degree. MQ has also been shown to inhibit the seeding promoted by mutant p53 cellular extracts fractions enriched in oligomers on WTP53C. The same seeding inhibition effect was observed for extracts from cells treated with PRIMA-1.

References

1. Muller PA, Vousden KH. p53 mutations in cancer. *Nat Cell Biol* 2013; 15:2-8; PMID:23263379; <http://dx.doi.org/10.1038/ncb2641>
2. Ano Bom AP, Rangel LP, Costa DC, de Oliveira GA, Sanches D, Braga CA, Gava LM, Ramos CH, Cepeda AO, Stumbo AC, et al. Mutant p53 aggregates into prion-like amyloid oligomers and fibrils: implications for cancer. *J Biol Chem* 2012; 287:28152-62; PMID:22715097; <http://dx.doi.org/10.1074/jbc.M112.340638>
3. Silva JL, Vieira TC, Gomes MP, Bom AP, Lima LM, Freitas MS, Ishimaru D, Cordeiro Y, Foguel D. Ligand binding and hydration in protein misfolding: insights from studies of prion and p53 tumor suppressor proteins. *Acc Chem Res* 2010; 43:271-9; PMID:19817406; <http://dx.doi.org/10.1021/ar900179t>
4. Ishimaru D, Andrade LR, Teixeira LS, Quesado PA, Maiolino LM, Lopez PM, Cordeiro Y, Costa LT, Heckl WM, Weissmüller G, et al. Fibrillar aggregates of the tumor suppressor p53 core domain. *Biochemistry* 2003; 42:9022-7; PMID:12885235; <http://dx.doi.org/10.1021/bi034218k>

5. Silva JL, Rangel LP, Costa DC, Cordeiro Y, De Moura Gallo CV. Expanding the prion concept to cancer biology: dominant-negative effect of aggregates of mutant p53 tumour suppressor. *Biosci Rep* 2013; 33:e00054; PMID: 24003888; <http://dx.doi.org/10.1042/BSR20130065>
6. Xu J, Reumers J, Couceiro JR, De Smet F, Gallardo R, Rudyak S, Cornelis A, Rozanski J, Zwolinska A, Marine JC, et al. Gain of function of mutant p53 by coaggregation with multiple tumor suppressors. *Nat Chem Biol* 2011; 7:285-95; PMID:21445056; <http://dx.doi.org/10.1038/nchembio.546>
7. Forget KJ, Tremblay G, Roucou X. p53 Aggregates penetrate cells and induce the co-aggregation of intracellular p53. *PLoS One* 2013; 8:e69242; PMID:23844254; <http://dx.doi.org/10.1371/journal.pone.0069242>
8. Lasagna-Reeves CA, Clos AL, Castillo-Carranza D, Sengupta U, Guerrero-Muñoz M, Kelly B, Wagner R, Kaye R. Dual role of p53 amyloid formation in cancer; loss of function and gain of toxicity. *Biochem Biophys Res Commun* 2013; 430:963-8; PMID:23261448; <http://dx.doi.org/10.1016/j.bbrc.2012.11.130>
9. Levy CB, Stumbo AC, Ano Bom AP, Portari EA, Cordeiro Y, Silva JL, De Moura-Gallo CV. Co-localization of mutant p53 and amyloid-like protein aggregates in breast tumors. *Int J Biochem Cell Biol* 2011; 43:60-4; PMID:21056685; <http://dx.doi.org/10.1016/j.biocel.2010.10.017>
10. Soto C. Transmissible proteins: expanding the prion heresy. *Cell* 2012; 149:968-77; PMID: 22632966; <http://dx.doi.org/10.1016/j.cell.2012.05.007>
11. Prusiner SB. Cell biology. A unifying role for prions in neurodegenerative diseases. *Science* 2012; 336:1511-3; PMID:22723400

P.234: Is the fatal familial insomnia mutation toxic due to disruption of the disulfide bridge?

Walker S Jackson

German Center for Neurodegenerative Diseases (DZNE); Bonn, Germany

One of the most remarkable features of human prion diseases is that different prion protein (PrP) mutants lead to very different familial disease forms, with different shapes of PrP aggregates, different degrees of spongiform degeneration, and different locations of neuronal loss and reactive gliosis. In recent years we have developed an allelic series of PrP knock-in mice to study how mutations confer toxic properties on PrP. We have recently compared and contrasted mice modeling fatal familial insomnia (FFI) and familial Creutzfeldt-Jakob disease (CJD). Both mutations caused neurodegenerative processes but the diseases were quite different. The FFI model had neuronal loss, reactive gliosis, and proteinase K sensitive PrP aggregates, predominantly in the thalamus, while the CJD model had spongiosis and proteinase K resistant PrP aggregates, most prominently in the hippocampus. Based on neuropathological and behavioral changes, we judged the FFI mutation to be more toxic. We hypothesized that the FFI mutant's higher toxicity was due to its proximity to one of the cysteines comprising PrP's sole disulfide bridge, thereby interfering with bridge formation and resulting in a destabilized and toxic species. In support of this conjecture, the steady state level of the FFI mutant PrP is reduced ~ 70%, consistent with most molecules not folding properly and being triaged for degradation soon after synthesis. We further hypothesized that complete elimination of the bridge via mutagenesis would result in a disease resembling that of the FFI model, but more severe. We tested this hypothesis by developing knock-in mice expressing PrP with both cysteines replaced with serines, which are structurally similar. These mice developed disease spontaneously, but surprisingly, it was more like that of the CJD model, with wide-spread spongiosis. In an effort to create a version with more severe disease we resorted to the commonly used random integration strategy to over-express

the same PrP mutant. Surprisingly, despite increased expression levels, the random integration mice were normal in all regards, notably lacking the spongiosis present in the knock-in line. Moreover, the random integration line was surprisingly effective at delaying scrapie infection, dominantly. These results indicate that the mechanisms of toxicity of mutant PrPs are difficult to predict and can be altered when PrP is expressed ectopically.