OPEN ACCESS Freely available online

PLOS ONE

PrP^{ST}, a Soluble, Protease Resistant and Truncated PrP Form Features in the Pathogenesis of a Genetic Prion Disease

Yael Friedman-Levi[ϑ], **Michal Mizrahi**[ϑ], **Kati Frid, Orli Binyamin, Ruth Gabizon***

Department of Neurology, The Agnes Ginges Center for Human Neurogenetics, Hadassah University Hospital, Jerusalem, Israel

Abstract

While the conversion of PrP^{C} into PrP^{Sc} in the transmissible form of prion disease requires a preexisting PrP^{Sc} seed, in genetic prion disease accumulation of disease related PrP could be associated with biochemical and metabolic modifications resulting from the designated PrP mutation. To investigate this possibility, we looked into the time related changes of PrP proteins in the brains of TgMHu2ME199K/wt mice, a line modeling for heterozygous genetic prion disease linked to the E200K PrP mutation. We found that while oligomeric entities of mutant E199KPrP exist at all ages, aggregates of wt PrP in the same brains presented only in advanced disease, indicating a late onset conversion process. We also show that most PK resistant PrP in TgMHu2ME199K mice is soluble and truncated (PrP^{ST}), a pathogenic form never before associated with prion disease. We next looked into brain samples from E200K patients and found that both PK resistant PrPs, PrP^{ST} as in TgMHu2ME199K mice, and "classical" PrP^{Sc} as in infectious prion diseases, coincide in the patient's post mortem brains. We hypothesize that aberrant metabolism of mutant PrPs may result in the formation of previously unknown forms of the prion protein and that these may be central for the fatal outcome of the genetic prion condition.

Citation: Friedman-Levi Y, Mizrahi M, Frid K, Binyamin O, Gabizon R (2013) PrP^{ST}, a Soluble, Protease Resistant and Truncated PrP Form Features in the Pathogenesis of a Genetic Prion Disease. PLoS ONE 8(7): e69583. doi:10.1371/journal.pone.0069583

Editor: Giovanna R. Mallucci, University of Leicester, United Kingdom

Received May 7, 2013; **Accepted** June 12, 2013; **Published** July 26, 2013

Funding: The work was done by a grant from the ISF foundation. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: gabizonr@hadassah.org.il

ϑ These authors contributed equally to this work.

Introduction

Detergent insoluble and PK resistant PrP, also known as PrP^{Sc}, was identified concomitantly with the enrichment of the prion agent in infected hamsters and mice [1]. Since then, PrP^{Sc} was established as the "gold standard" marker of prion infection and its presence in scrapie infected sheep, BSE infected cattle and in humans suffering from Creutzfeldt-Jacob disease (CJD) was used to confirm a prion disease diagnosis [2,3], while in its absence such a verdict is mostly ruled out [4,5,6]. However, classical PrP^{Sc} could not be detected in brains of patients suffering from some forms of genetic prion diseases [5,7,8], which are autosomal dominant disorders linked to mutations in the gene encoding the prion protein (PrP) [9] [10]. PrP^{Sc} is also undetected or present at marginal levels in the brains of most transgenic mice modeling for genetic prion diseases [11] [12] [13] [14] [15] [16].

The most common genetic CJD (gCJD) is the one linked to the E200K PrP mutation (substituting lysine for glutamate) [17,18]. This mutation was identified among Jews of Libyan origin as well as in subjects of other communities around the world [19]. Not only is E200K CJD relatively frequent, but is also the gCJD most similar to sporadic CJD in age of onset, clinical and pathological presentation, as well as in the accumulation of classical PrP^{Sc} in the brains of the affected subjects [20]. While most E200K CJD patients are heterozygous for the mutation, disease presentation was faster to some degree in a small number of homozygous patients [21].

To investigate whether the presence of an E200K mutation is enough to confer high levels of protease resistance and aggregation to the nascent mutant prion protein, E200K PrP in cell cultured models was investigated by diverse biochemical methods. It was found to be either readily digested by PK or otherwise resistant to marginal PK concentrations [22,23]. Interestingly, E200K PrP in recombinant form is spontaneously oxidized in its helix 3 methionine residues, a covalent modification which precedes the conversion of PrP^{C} into PrP^{Sc} [24]. All this suggests that while E200K PrP presents intrinsic properties in between those of PrP^{C} and PrP^{Sc}, its full conversion into a disease related form may occur only in-vivo in an age dependent form, concomitant with the late onset nature of the disease.

Our knowledge on levels and properties of disease related mutant PrPs in genetic prion diseases was gathered mostly from post mortem samples [25]. To investigate the changes in mutant PrP properties at different time points in a late onset disease setup, we looked into the biochemical properties of PrP in brain samples of TgMHu2ME199K mice. These mice, which model for E200K CJD, express human- mouse chimeric E199K PrP on a null (for homozygous) or a wt PrP (for heterozygous) background. Mice from both lines suffer from similar neurological symptoms from as early as 5–6 month of age and deteriorated to a terminal condition several months thereafter. During disease, TgMHu2ME199K

mice accumulate a truncated form of PK resistant PrP recognizable by C-terminal PrP antibodies [26]. Like in the brains of human E200K patients [27,28], infectious prions are spontaneously formed in TgMHu2ME199K mice, mostly in the sick TgMHu$_2$ME200K/wt mice, and only rarely in the sick TgMHu2-ME199K/ko, suggesting that the presence of a wt allele, while not required for disease presentation, may facilitate the transmission of infectivity to wt mice.

We now show that E199K PrP in TgMHu2ME199K mice present in oligomeric forms long before disease presentation, indicating partial aggregation, as oxidation, is an intrinsic property of this mutant PrP. Contrarily, PK resistant PrP is detected just before disease presentation and accumulates further with disease aggravation, indicating its formation is an age related effect. Wt PrP aggregates can be identified in brains of heterozygous Tg mice only when disease is well established, indicating the conversion of wt PrP to a disease like form is an acquired property related to the status of the disease. Surprisingly, results from brain fractionation experiments demonstrate that the truncated PK resistant PrP in the TgMHu2ME199K mice present in soluble brain fractions, as was recently suggested for pathological key proteins in other neurodegenerative conditions [29,30,31]. Most important, we found that both disease related PrP forms, the soluble, truncated and PK resistant, hereby denominated PrPST as well as the "classical" PrP^{Sc}, coincide in the brains of E200K patients. We hypothesize that the metabolism of mutant PrP may result in the formation of previously unknown PrP forms, some of which may be essential for the fatal outcome of the disease while others may be more active in disease transmission.

Results

Clinical disease and PrP accumulation in heterozygous and homozygous TgMH2ME199K mice

Except for rare cases related to family intermarriages [21], most genetic CJD patients are heterozygous for the mutation, thereby expressing both mutant and wt PrP [32]. While we have shown previously that TgMHu2ME199K mice on a null or wt background present similar disease properties and kinetics [26], we looked again at this observation by calculating the individual time points in which each mouse reaches score 2, a clear observation point in disease aggravation (significant hind limb/s weakness). Using this calculation method, we show (fig. 1a) that the median of disease aggravation is indeed very similar for both lines; 5.2 months $\pm$0.8 for homozygous mice and 6.0$\pm$1.2 months for heterozygous mice. Interestingly, there is no statistical significant difference between the kinetics of aggravation in both lines of mice (0.37 by T-test), indicating that a wt PrP allele is not a must for disease presentation and progression. Consistent with these results, figure 1b shows that the levels and rate of PK resistant PrP accumulation in the brain homogenates of TgMHu2ME199K/ko and TgMHu2ME199K/wt mice are very similar, suggesting again that wt PrP in genetic patients is not an obligatory part of the pathological process [33]. As published before, PK resistant PrP in these mice present as a truncated form recognizable only by C-terminal αPrP antibodies [24,26], as the pAb RTC used in figure 1b, which cannot separate between the chimeric and wt allele.

E199K PrP as compared to wt PrP aggregation properties

As opposed to the lack of a role for wt PrP in the onset and progression of genetic prion disease, this is not the case for disease transmission to naïve animals. We have shown previously [24] that while brains from sick TgMHu2ME199K/wt mice readily transmitted disease to wt mice, only rare mice succumb to disease when inoculated with samples from asymptomatic heterozygous brains or even from sick TgMHu2ME199K/ko brains. This indicates that some levels of wt PrP in heterozygous TgMHu2-ME199K mice may convert into infectious prions when disease is well established.

Since we could not separate between truncated allele specific PK resistant PrP due to the lack of appropriate antibodies, we looked into the aggregation properties of full length wt and mutant PrP at different disease points. To this effect, we subjected sarkosyl extracted brain homogenates of TgMHu2ME199K and wt mice to sucrose gradients that can separate between aggregates of different sizes and solubilized PrP forms. Fractions from these gradients were subjected to immunoblotting with α PrP antibodies that can distinguish between wt and chimeric mouse-human E199K PrP when at full length. Panel a in figure 2 describes the PrP epitopes of all antibodies used in this manuscript. Indeed, α PrP mAb IPC1 recognizes murine PrP but not chimeric MHu2M forms, while αPrP mAb 3F4 recognizes the human parts of chimeric E199K PrP, but not murine PrP as in the wt allele. In panel b, we show that PrP in wt mice, as recognized by IPC1, is present only in the low density fractions of the gradient, as expected for PrP^{C}, a membrane protein that is readily solubilized in detergents. The same results (not shown) were obtained for wt PrP in mice brains of all ages. Contrarily, PrP in the brains of TgMHu2ME199K/ko mice (as detected by αPrP mAb 3F4), is dispersed between all gradient fractions, indicating it may intrinsically form oligomers of different sizes. Next, we looked into the aggregation properties of both PrPs in TgMHu2-ME199K/wt mice of different ages, before and after disease presentations. Fig. 2c demonstrates that while E199K PrP presents the same oligomerization levels from 1 month old mice through all ages, wt PrP in the heterozygous mice is solubilized at young age, as is the case for PrP^{C} in wt mice, but acquires aggregation properties when clinical disease become apparent at older age, as is the case for 7 month old TgMHu2ME199K mice. These results suggest an "in cell" infection process leading to the conversion of wt PrP into a disease related form. As stated above, whether some of this aggregated wt PrP forms are also PK resistant cannot be determined by these tools.

Truncated PK resistant PrP is present in the light fractions of TgMHu2ME199K brains gradients

In the brains of rodents affected with scrapie, PrP^{Sc} is present only in the most aggregated fractions of a sucrose gradient [34]. To test whether this is also the case for PrP in TgMHu2ME199K/wt mice, we digested gradients samples in the presence or absence of PK and immunoblotted then either with α PrP mAb 6H4 (for full length PrP) or α PrP pAbRTC (total+truncated). Figure 3 shows that, as described for the TgMHu2ME199K/ko [26], PK resistant PrP in these mice can only be identified by pAb RTC (α C-terminal PrP) and not by 6H4. However, and contrarily to brains of prion infected mice, most PK resistant PrP in the brains of the TgMHu2ME199K mice, both of wt (fig. 3) or ablated (not shown) background, could be detected in the light fractions, representing either a soluble protein or a detergent solubilized membrane protein. This is a surprising result not only due to the different properties of PrP^{c} in the TgMHu2ME199K mice as compared to classical PrP^{Sc} in transmissible prion disease, but also in view of the intrinsic aggregation properties of this mutant PrP. We speculate that degradation of aberrantly folded mutant PrP may result also in soluble truncated products, each of them with different biochemical properties.

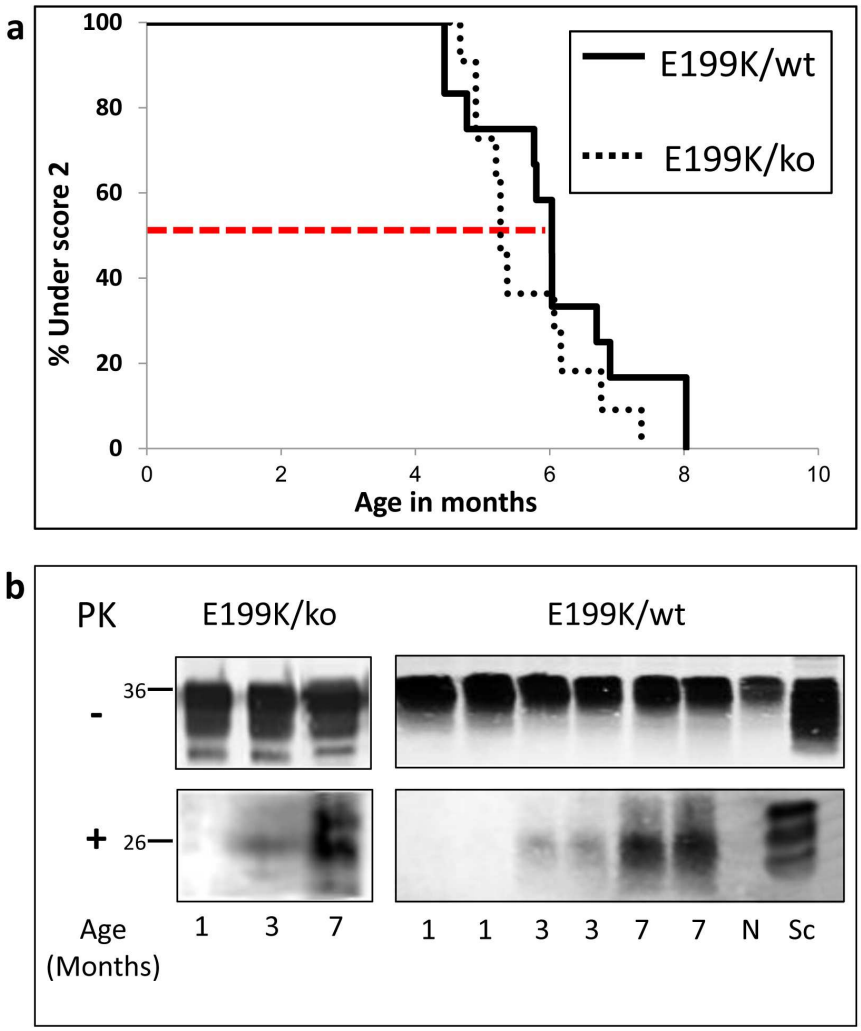

Figure 1. Similar disease kinetics and PrP accumulation in heterozygous and homozygous TgMH2ME199K mice. (**a**) The percentage of mice presenting a score <2 in both E199K/ko and E199K/wt lines as related to age. Median was 5.2±0.8 for TgMHu2ME199K/ko mice and 6.0±1.2 for TgMHu2ME199K/wt mice. (**b**) PK-resistant PrP levels are similar in both lines as related to age. Brain homogenates from both TgMHu2ME199K/KO and TgMHu2ME199K/wt lines in different ages and clinical stages (1 month, 3 months and 7 months, score = 0, score = 0 score = 3 respectively) as well as wt and scrapie RML controls were digested with PK and immunoblotted with α-PrP pAb RTC.
doi:10.1371/journal.pone.0069583.g001

Truncated PrP in human gCJD brain samples

To test whether truncated forms of PrP as described for the TgMHu2ME199K mice also feature in the human relevant disease, sucrose gradients as above were loaded with sarkosyl extracted samples of E200K CJD brains, originating from post mortem samples. Fractions collected from these gradients were digested in the presence or absence of PK and immunoblotted with αPrP mAb 6H4 and with αPrP pAb RTC, as done above for the TgMHu2ME199K samples. Figure 4 shows that while α PrP mAb 6H4 detects classical forms of PrP^{Sc} in the heavy fractions of the gradient after proteolysis, pAb RTC also detects soluble truncated forms of PrP before and after PK digestion, albeit with less intensity than the ones observed in the PK treated TgMHu2ME199K brains. The fact that truncated PK resistant forms of PrP could be found in the light fraction of both E200K human patients and sick TgMHu2ME199K mice indicates this form of PrP most probably plays a role in the pathological process leading to fatal disease in genetic cases, the nature of which is still unknown. Regretfully, and since we can only look into an "end point" situation in the human samples, it is difficult to determine whether "classical PrP^{Sc}" starts to accumulate in these patients at an early or late stage in the disease process. Also in the TgMHu2ME199K mice we are lacking part of the time frame spectrum since our ethical permit does not allow us to follow up very sick mice for long periods of time, but rather we have to sacrifice them when they cannot reach by themselves to food and water.

PrP^{ST}: A truncated, PK resistant and soluble disease related PrP

Disease related PrP forms presenting in light fractions of brain homogenate gradients may be membrane proteins solubilized in sarkosyl or bona fide soluble proteins. To distinguish between these possibilities, we subjected samples from E200K CJD patients and from TgMHu2ME199K/wt mice, as well as brains samples from naïve and scrapie RML infected mice to the following fractionation protocol (see figure 5a). First, brain homogenates (see methods for details), after a 18000 rpm centrifugation were

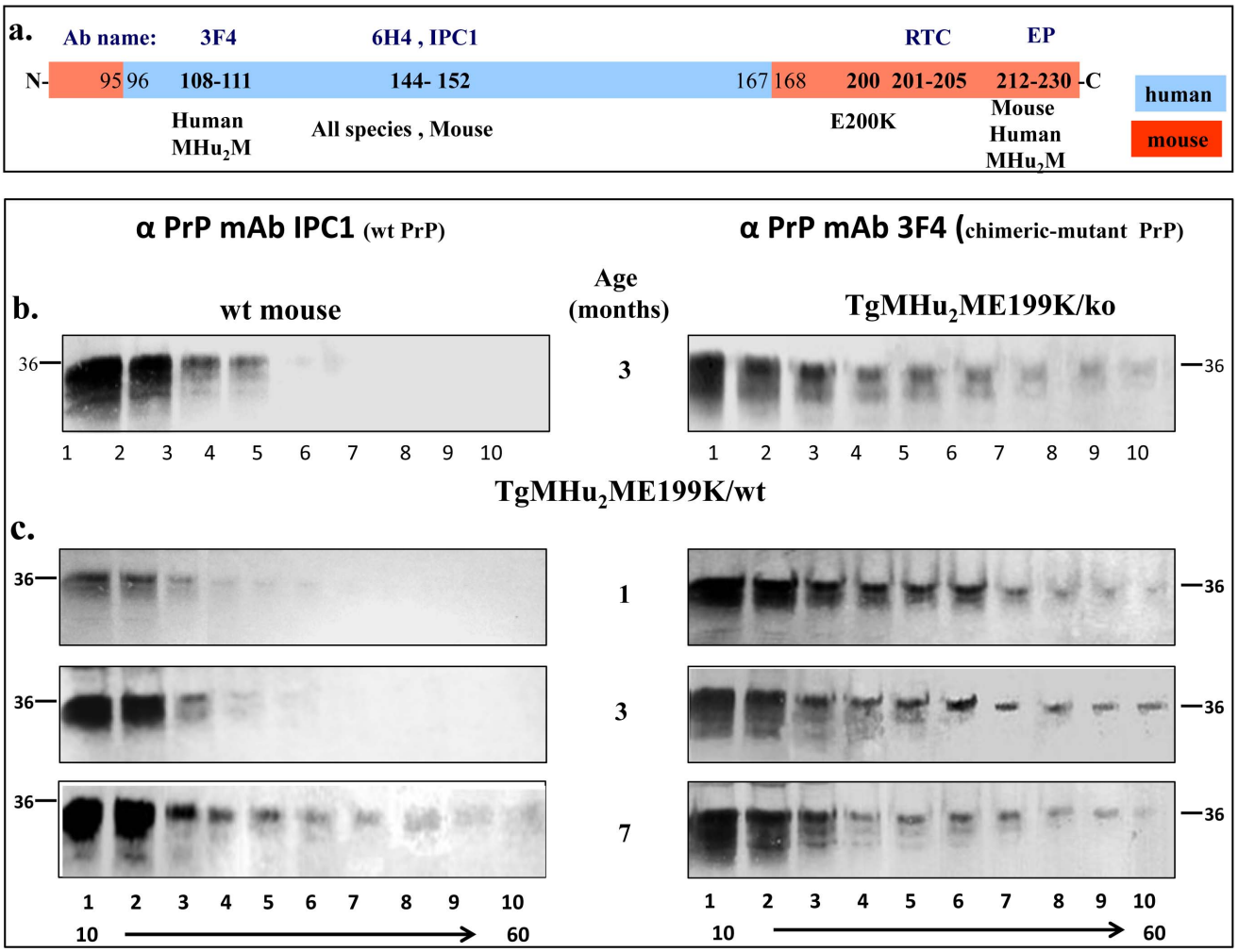

Figure 2. Aggregation of wt and mutant PrP in TgMHu2ME199K/wt mice. (**a**) **Epitope mapping of α-PrP antibodies:** epitopes of antibodies used in this manuscript are depicted on a schematic representation of the chimeric mouse-human E199K PrP. In the next panels (b &c) αPrP mAbs IPC1 and 3F4 were used to differentiate between wt PrP and chimeric-mutant PrP respectively. (**b**) **Oligomeric E199K PrP in asymptomatic TgMHu2ME199K/ko mice:** Sarkosyl extracted brain homogenates of wt and 3 months old (score = 0) TgMHu2ME199K/ko mouse were subjected to ultracentrifugation in 10–60% sucrose gradients [34]. Individual fractions were immunoblotted with αPrP mAb IPC1 to detect wt PrP and αPrP mAb 3F4 to detect chimeric-mutant PrP. (**c**) **Oligomeric wt PrP in sick TgMHu2ME199K/wt mice.** Sarkosyl extracted brain homogenates from TgMHu2ME199K/wt mice at different ages (1 month, 3 months and 7 months, score = 0, score = 0 score = 3 respectively) were subjected to ultracentrifugation in 10–60% sucrose gradients. Individual fractions of each gradient were immunoblotted with mAb IPC1 to detect wt PrP and mAb 3F4 to detect chimeric-mutant PrP.
doi:10.1371/journal.pone.0069583.g002

separated into pellet and supernatant. To obtain a clear supernatant carrying only soluble proteins, this first supernatant was subjected twice to ultracentrifugation at 100000 g. The supernatant of the last centrifugation was digested in the presence or absence of PK. In parallel the first pellet was subjected to osmotic shock before ultracentrifugation (to eliminate traces of supernatant material) and subsequently the pellet was solubilized in the presence of 2% sarkosyl and centrifuged at 100000 g. The pellet and supernatant of this last procedure were digested in the presence and absence of PK. Finally, the sarkosyl pellet and supernatants, as well as the high speed soluble fractions were immunoblotted with two C-terminal α PrP antibodies; pAb RTC and EP1802Y (EP) , a rabbit α PrP mAb directed against the CITQYER ESQAYYQRGS sequence present at the C-terminal part of human PrP, just before the PrP GPI anchor. All PK digestions were performed in the presence of 2% sarkosyl, to ensure the accessibility to the protease in case of protein membrane interactions.

Results of these experiments are depicted in Figure 5b&c. surprisingly, figure 2b shows that significant levels of PrP are present in soluble fractions of all brains, as opposed to the general notion that the prion proteins are mostly associated with membranes. However, only in the genetic disease related samples, E200K human brains and TgMHu2ME199K brains, are these soluble PrP forms resistant to PK digestion in a truncated form. Interestingly, some of the PrP^{ST} forms are recognized only by RTC and not by the EP antibody, indicating they may be truncated not only in its N-terminal but also in its C-terminal part. Most important, no PrP^{ST} form was found in the soluble brain fractions of scrapie infected mice, indicating that genetic and transmissible prion disease differ in some mechanistic features.

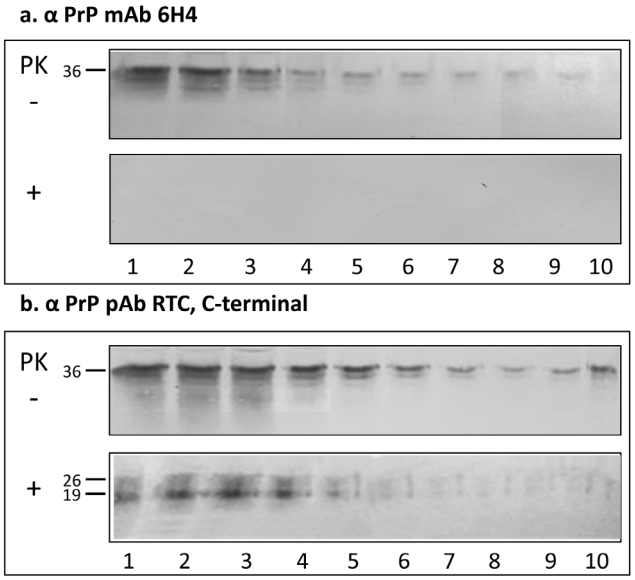

Figure 3. PK-resistant PrP in light fractions of TgMHu2ME199K brain gradients. Sarkosyl extracted brain homogenates of sick TgMHu2ME199K/wt mice (in the figure: 9 month old score = 4) were subjected to ultracentrifugation in 10–60% sucrose gradients. Individual fractions were digested in the presence or absence of PK and immunoblotted with: (**a**) α-PrP mAb 6H4 and (**b**) α-PrP pAb RTC directed against the C-terminal end of PrP.
doi:10.1371/journal.pone.0069583.g003

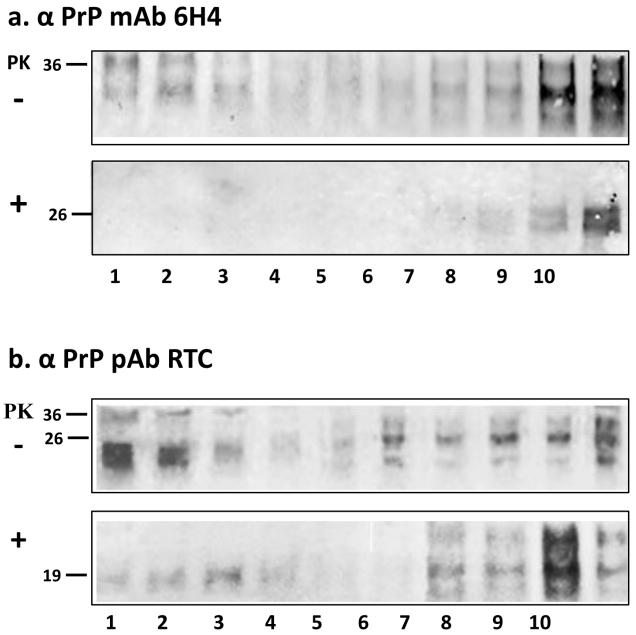

Figure 4. Disease related PrP forms in brains from E200K gCJD patients. Sarkosyl extracted brain homogenates from E200K CJD patients were subjected to ultracentrifugation in 10–60% sucrose gradients. Individual fractions were digested with PK and immunoblotted with: (a) α-PrP mAb 6H4 and (b) α-PrP pAb RTC.
doi:10.1371/journal.pone.0069583.g004

While it is difficult to establish whether PrP^{ST} is a soluble cytosolic protein or otherwise a soluble protein secreted into the intercellular space, most data suggests PrP metabolites in TgMHu2ME199K mice are intracellular. This was shown by α PrP immunohistochemistry in TgMHu2ME199K brains and TgMHu2ME199K primary fibroblasts [26,35], which show extensive intra cell immunostaining. In addition, we were unable to identify PrP^{ST} in the CSF of E200K patients (not shown). However, our results do not rule out the possibility that some levels of PrP^{C} and PrP^{ST} are secreted indeed into the intercellular space.

Only the TgMHu2ME199K mice show PrP^{ST} forms in the sarkosyl soluble fractions. Whether this relates to traces of the cytosolic form, or otherwise demonstrates that PrP^{ST} is formed on cell membranes and is shaded to the inside or the outside of cells after cutting its GPI anchor is unknown [36]. Interestingly, all but normal brain samples present PK resistant PrP in the sarkosyl extracted pellets each of those with a different pattern and at low levels at the TgMHu2ME199K mice. We may therefore conclude that while the transmissible form of prion disease (RML) shows only classical PrP^{Sc}, the genetically affected brains also present a unique soluble form, PrP^{ST} , which in the Tg mice constitutes the great majority of disease related PrP. We hypothesize that PrP^{ST} is the product of aberrant E200K PrP metabolism as related to age factors and its accumulation may be an important feature in the pathological mechanism of genetic forms of prion disease.

Discussion

Taking advantage of our TgMHu2ME199K/wt model of heterozygous E200K gCJD, we looked into the time related changes in the biochemical properties of E199K and wt PrP from the asymptomatic early age to full blown disease. We found that the disease burden lies fully on mutant PrP, since the addition of a wt allele did not affect disease time course or levels of PK resistant PrP accumulation. In addition, we established that some of the E199K PrP presents intrinsically in oligomeric forms even in the early asymptomatic stages, while the accumulation of a soluble truncated and PK resistant form (PrP^{ST}), most probably cytosolic, commences shortly before disease manifestation. Contrarily, wt PrP in the heterozygous mice maintains PrP^{C} like properties until much later in the life of the mice and starts to aggregate at 7 months of age, when disease is already at an advanced stage.

Most important, we show that brains of human E200K gCJD patients comprise both classical PrP^{Sc} and PrP^{ST}, the last one recognizable only by C-terminal α PrP antibodies. Since our TgMHu2ME199K mice succumb to neurological fatal disease, and PrP^{ST} is by far the major disease related PrP form accumulated in these animals, we speculate that PrP^{ST} may be the key feature of this genetic form of prion disease. Accumulation of classical PrP^{Sc} may be a late effect induced by the conversion of wt PrP in heterozygous patients. However, while in one homozygous E200K CJD patient, classical PrP^{Sc} could not be detected [21], we cannot conclude at this point that the accumulation of classical PrP^{Sc} in genetic patients only appears when wt PrP is expressed. It will be interesting to test whether this or other forms of PrP^{ST} are present in patients carrying other pathogenic PrP mutations as well as in sporadic CJD patients, each of which may show distinct PrP accumulation properties.

The generation of PrP^{ST} may result from the aberrant folding and abnormal metabolism and degradation of E200K PrP. We have shown previously that E200K PrP is spontaneously oxidized in its helix 3 Met residues [24], probably due to an increase in solvent exposure of the relevant helix in the presence of K at position 200 [37]. Such oxidation may provide the structural changes required for the formation of oligomeric and soluble disease related forms, such as PrP^{ST}. Cytosolic PrP^{ST} may next activate the UPR system, resulting in the global reduction in protein synthesis and dysregulation of elF2a [38]. In addition, we

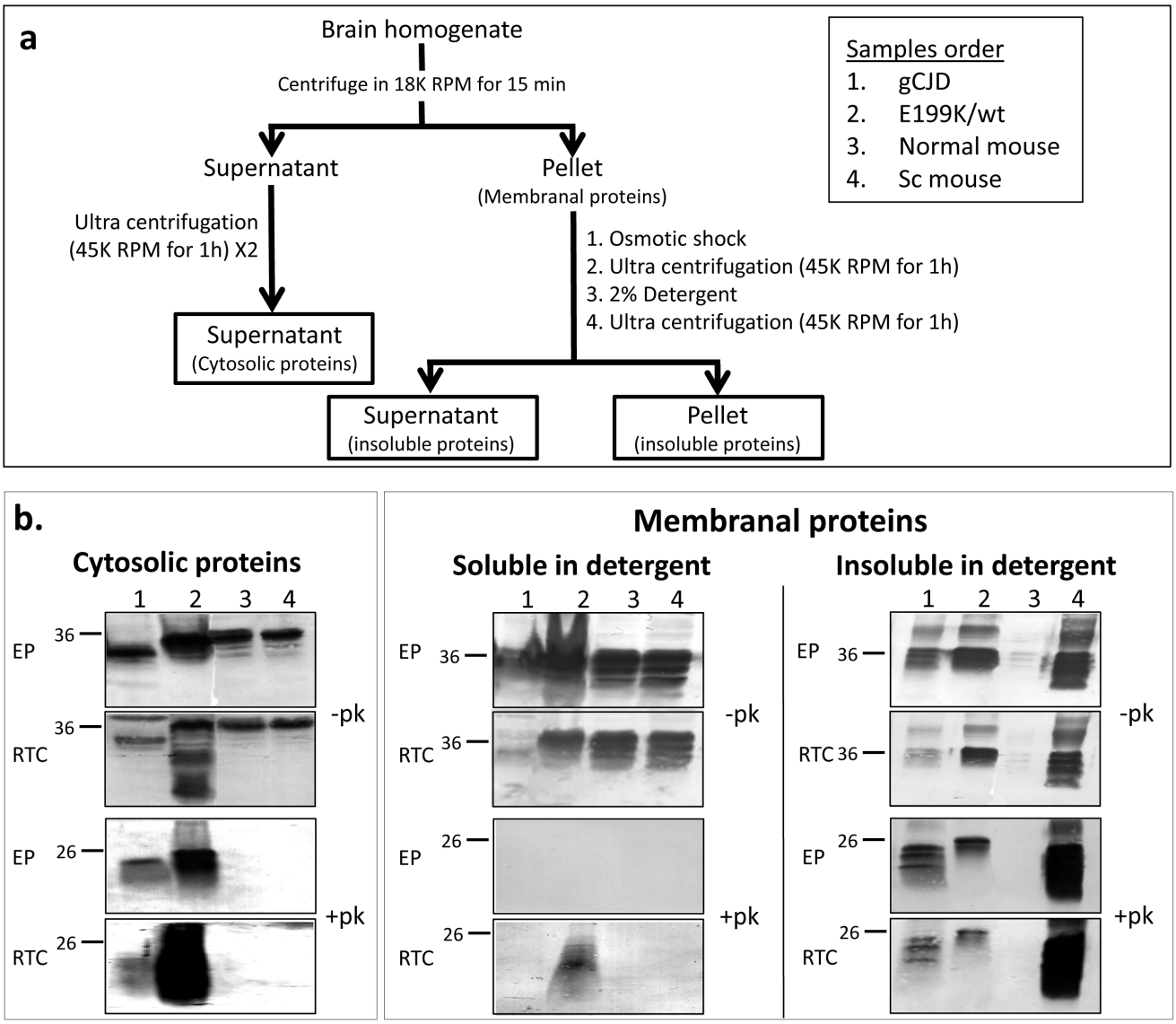

Figure 5. PrP forms in soluble and membranal brain fractions. (**a**) Fractionation protocol of brain homogenates. (**b**) Soluble and membranal brain fractions were digested in the presence and absence of PK and immunoblotted with two α-PrP c-terminal Ab; pAb RTC and mAb EP1802Y (EP) (see figure 2a for epitope mapping).
doi:10.1371/journal.pone.0069583.g005

have recently shown that the expression of Snord 3A, a transcript relevant to the activation of another of the UPR arms, is elevated with time and disease aggravation in TgMHu2ME199K mice, as is the case for humans suffering from gCJD [39].

Interestingly, soluble, as opposed to fibrillary forms of key pathogenic proteins were recently shown to be the culprit of disease in other neurodegenerative conditions, as is the case for A-beta [29] [40] and Tau [41]. Indeed, it is the levels of soluble Tau and not of Tau incorporated into neurofibrillary tangles which reduction led to the cessation of neuron loss and the improvement of memory functions [41]. Also, the removal of amyloid plaques by Aβ immunotherapy failed to improve the clinical status of Alzheimer patients [42]. If a similar mechanism exists for prion diseases, it is mostly PrP^{ST} and less so classical PrP^{Sc} which may be the key neurotoxic entity, at least in gCJD linked to the E200K PrP mutation.

In addition to an unknown number of subclinical BSE cases, a significant number of people at risk to develop prion disease are carriers of pathogenic PrP mutations. We have shown here that the key pathogenic events may differ significantly between transmissible and genetic prion diseases. While the first one may relate mostly to the process converting PrP^{C} to classical PrP^{Sc}, mutant PrP forms may generate metabolic abnormalities form early age, resulting in activation of stress related UPR signals and accumulation of PrP^{ST}, a soluble PK resistant PrP form. This implies that treatments for genetic patients and even more so prevention of disease manifestation for individuals at genetic prion risk cannot be evaluated only by disappearance of classical PrP^{Sc} from cells in culture or even by delay of disease onset in scrapie infected rodents, but should rather be tested in appropriate transgenic models that mimic for genetic prion diseases.

Materials and Methods

Ethical statement

Animal experiments were conducted under the guidelines and supervision of the Hebrew University Ethical Committee, which approved of the methods employed in this project (Permit Number: MD-11746-5). Brain human samples were received following postmortem examinations from the Pathology Departments of several hospitals in the country. Immunoblotting experiments on such samples, as the ones described in this manuscript, are part of the routine pathological protocol applied on brains from suspected CJD cases. Our laboratory in the Hadassah Department of Neurology is the national referral center for CJD diagnosis (genetic and biochemical testing). The testing of these samples was approved by both the safety and ethical authorities of the Hadassah University Hospital. Since all cases of CJD and alike negative controls are unable to sign for such tests long before their death due to their medical condition, the relatives of these patients provided informed written consent for PM studies. Enabling close relatives to provide such consent is the standard policy of the Israeli Ministry of Health.

Mice scoring

TgMHu2ME199K mice from both lines (PrP ablated or wt backgrounds) were followed twice a week for the appearance of spontaneous neurological disease. Mice were scored for disease severity and progression according to the scale of clinical signs as previously described [26]. Briefly, partial hind limbs weakness = 1, significant hind limb/s weakness or paralysis = 2, full paralysis in one limb = 3, full paralysis in both limbs = 4, death = 5. Mice were sacrificed according to the ethical requirements of the Hebrew University Animal Authorities when too sick or paralyzed to reach food and water, or after losing 20% body weight.

Western blot analysis

Brains were homogenized at 10% (W/V) in 10 mM Tris- HCl, pH 7.4 and 0.3 M sucrose. For Proteinase K digestions, 30 µl of 10% brain homogenates extracted with 2% sarkosyl on ice were incubated with 30 to 40 mg/ml Proteinase K for 30 min at 37°C. Samples were subsequently subjected to SDS PAGE and immunoblotted with diverse anti-PrP antibodies, as described in Figure 2a.

Sucrose gradients of brain homogenates

Sarkosyl extracted brain homogenates were subjected to sucrose gradients as described [34]. Briefly, 140 to 300 µl of 10% brain homogenates extracted in the presence of 2% Sarkosyl were overlaid on a sucrose gradient composed of layers of increasing concentrations of sucrose (10–60%). Gradients were then centrifuged for 1 h at 55000 rpm in a Sorval mini-ultracentrifuge and subsequently 9 samples were collected from the top to the bottom. 2/3 volume of each individual sample was then subjected to Proteinase K digestion and immunoblotted with diverse anti PrP Abs.

Brain Fractionation

Two ml of 10% brain homogenates in 10 mM Tris-HCl, pH 7.4/0.3M sucrose from human E200K gCJD patients, 8 months old transgenic E199K/wt mouse, 8 months old normal mice and scrapie RML mice at terminal stages of disease were subjected to centrifugation at 18,000 rpm for 15 minutes at 4°C. Subsequently, 300 µl of each supernatant was subjected twice to ultra-centrifugation (45,000 rpm for 1 hour at 4°C) in order to eliminate traces of membranes from the soluble protein fraction. Proteins in the last supernatant were concentrated by methanol precipitation, and then resuspended in STE (saline-*tris-EDTA*) (fraction I). The initial pellet containing membrane proteins was resuspended in DDW and ultra-centrifuged to dilute traces of supernatant material, and the pellet resuspended and extracted in 2% sarkosyl before ultra-centrifugation in 45,000 rpm for 1 hour at 4°C. The detergent soluble fraction (II) and the pellet of membrane proteins (III), together with fraction I were digested in the presence or absence of 40 ug/ml PK and immunoblotted with C-terminal αPrP antibodies.

Author Contributions

Conceived and designed the experiments: YF MM RG . Performed the experiments: YF MM KF OB. Analyzed the data: YF MM RG. Wrote the paper: YF MM RG.

References

1. Bolton DC, McKinley MP, Prusiner SB (1982) Identification of a protein that purifies with the scrapie prion. Science 218: 1309–1311.
2. Mohri S, Farquhar CF, Somerville RA, Jeffrey M, Foster J, et al. (1992) Immunodetection of a disease specific PrP fraction in scrapie-affected sheep and BSE-affected cattle. Vet Rec 131: 537–539.
3. Prusiner SB, Hsiao KK (1994) Human prion diseases. Ann Neurol 35: 385–395.
4. Gambetti P, Kong Q, Zou W, Parchi P, Chen SG (2003) Sporadic and familial CJD: classification and characterisation. Br Med Bull 66: 213–239.
5. Xiao X, Cali I, Dong Z, Puoti G, Yuan J, et al. (2013) Protease-sensitive prions with 144-bp insertion mutations. Aging (Albany NY) 5: 155–173.
6. Varges D, Schulz-Schaeffer WJ, Wemheuer WM, Damman I, Schmitz M, et al. (2013) Spongiform encephalopathy in siblings with no evidence of protease-resistant prion protein or a mutation in the prion protein gene. J Neurol.
7. Monaco S, Fiorini M, Farinazzo A, Ferrari S, Gelati M, et al. (2012) Allelic origin of protease-sensitive and protease-resistant prion protein isoforms in Gerstmann-Straussler-Scheinker disease with the P102L mutation. PLoS One 7: e32382.
8. Kim C, Haldiman T, Cohen Y, Chen W, Blevins J, et al. (2011) Protease-sensitive conformers in broad spectrum of distinct PrPSc structures in sporadic Creutzfeldt-Jakob disease are indicator of progression rate. PLoS Pathog 7: e1002242.
9. Kovacs GG, Puopolo M, Ladogana A, Pocchiari M, Budka H, et al. (2005) Genetic prion disease: the EUROCJD experience. Hum Genet 118: 166–174.
10. Hsiao K, Prusiner SB (1990) Inherited human prion diseases. Neurology 40: 1820–1827.
11. Hsiao KK, Scott M, Foster D, Groth DF, DeArmond SJ, et al. (1990) Spontaneous neurodegeneration in transgenic mice with mutant prion protein. Science 250: 1587–1590.
12. Harris DA, Chiesa R, Drisaldi B, Quaglio E, Migheli A, et al. (2000) A transgenic model of a familial prion disease. Arch Virol Suppl: 103–112.
13. Dossena S, Imeri L, Mangieri M, Garofoli A, Ferrari L, et al. (2008) Mutant prion protein expression causes motor and memory deficits and abnormal sleep patterns in a transgenic mouse model. Neuron 60: 598–609.
14. Jackson WS, Borkowski AW, Faas H, Steele AD, King OD, et al. (2009) Spontaneous generation of prion infectivity in fatal familial insomnia knockin mice. Neuron 63: 438–450.
15. Asante EA, Gowland I, Grimshaw A, Linehan JM, Smidak M, et al. (2009) Absence of spontaneous disease and comparative prion susceptibility of transgenic mice expressing mutant human prion proteins. J Gen Virol 90: 546–558.
16. Telling GC, Haga T, Torchia M, Tremblay P, DeArmond SJ, et al. (1996) Interactions between wild-type and mutant prion proteins modulate neurodegeneration in transgenic mice. Genes Dev 10: 1736–1750.
17. Hsiao K, Meiner Z, Kahana E, Cass C, Kahana I, et al. (1991) Mutation of the prion protein in Libyan Jews with Creutzfeldt-Jakob disease. N Engl J Med 324: 1091–1097.
18. Korczyn AD, Chapman J, Goldfarb LG, Brown P, Gajdusek DC (1991) A mutation in the prion protein gene in Creutzfeldt-Jakob disease in Jewish patients of Libyan, Greek, and Tunisian origin. Ann N Y Acad Sci 640: 171–176.
19. Lee HS, Sambuughin N, Cervenakova L, Chapman J, Pocchiari M, et al. (1999) Ancestral origins and worldwide distribution of the PRNP 200K mutation causing familial Creutzfeldt-Jakob disease. Am J Hum Genet 64: 1063–1070.
20. Meiner Z, Kahana E, Baitcher F, Korczyn AD, Chapman J, et al. (2011) Tau and 14-3-3 of genetic and sporadic Creutzfeldt-Jakob disease patients in Israel. J Neurol 258: 255–262.

21. Simon ES, Kahana E, Chapman J, Treves TA, Gabizon R, et al. (2000) Creutzfeldt-Jakob disease profile in patients homozygous for the PRNP E200K mutation. Ann Neurol 47: 257–260.
22. Rosenmann H, Talmor G, Halimi M, Yanai A, Gabizon R, et al. (2001) Prion protein with an E200K mutation displays properties similar to those of the cellular isoform PrP(C). J Neurochem 76: 1654–1662.
23. Lehmann S, Harris DA (1996) Two mutant prion proteins expressed in cultured cells acquire biochemical properties reminiscent of the scrapie isoform. Proc Natl Acad Sci U S A 93: 5610–5614.
24. Canello T, Frid K, Gabizon R, Lisa S, Friedler A, et al. (2010) Oxidation of Helix-3 methionines precedes the formation of PK resistant PrP. PLoS Pathog 6: e1000977.
25. Cali I, Castellani R, Yuan J, Al-Shekhlee A, Cohen ML, et al. (2006) Classification of sporadic Creutzfeldt-Jakob disease revisited. Brain 129: 2266–2277.
26. Friedman-Levi Y, Meiner Z, Canello T, Frid K, Kovacs GG, et al. (2011) Fatal Prion Disease in a Mouse Model of Genetic E200K Creutzfeldt-Jakob Disease. PLoS Pathog 7: e1002350.
27. Telling GC, Scott M, Mastrianni J, Gabizon R, Torchia M, et al. (1995) Prion propagation in mice expressing human and chimeric PrP transgenes implicates the interaction of cellular PrP with another protein. Cell 83: 79–90.
28. Tateishi J, Kitamoto T (1995) Inherited prion diseases and transmission to rodents. Brain Pathol 5: 53–59.
29. Ashe KH, Aguzzi A (2012) Prions, prionoids and pathogenic proteins in Alzheimer disease. Prion 7: 55–59.
30. Lesne SE, Sherman MA, Grant M, Kuskowski M, Schneider JA, et al. (2013) Brain amyloid-beta oligomers in ageing and Alzheimer's disease. Brain.
31. Ma QL, Zuo X, Yang F, Ubeda OJ, Gant DJ, et al. (2013) Curcumin suppresses soluble tau dimers and corrects molecular chaperone, synaptic, and behavioral deficits in aged human tau transgenic mice. J Biol Chem 288: 4056–4065.
32. Gabizon R, Telling G, Meiner Z, Halimi M, Kahana I, et al. (1996) Insoluble wild-type and protease-resistant mutant prion protein in brains of patients with inherited prion disease. Nat Med 2: 59–64.
33. Chen SG, Parchi P, Brown P, Capellari S, Zou W, et al. (1997) Allelic origin of the abnormal prion protein isoform in familial prion diseases. Nat Med 3(9): 1009–1015.
34. Tzaban S, Friedlander G, Schonberger O, Horonchik L, Yedidia Y, et al. (2002) Protease-sensitive scrapie prion protein in aggregates of heterogeneous sizes. Biochemistry 41: 12868–12875.
35. Canello T, Friedman-Levi Y, Mizrahi M, Binyamin O, Cohen E, et al. (2012) Copper is toxic to PrP-ablated mice and exacerbates disease in a mouse model of E200K genetic prion disease. Neurobiol Dis 45: 1010–1017.
36. Stahl N, Borchelt DR, Hsiao K, Prusiner SB (1987) Scrapie prion protein contains a phosphatidylinositol glycolipid. Cell 51: 229–240.
37. Meli M, Gasset M, Colombo G (2012) Dynamic diagnosis of familial prion diseases supports the beta2-alpha2 loop as a universal interference target. PLoS One 6: e19093.
38. Moreno JA, Radford H, Peretti D, Steinert JR, Verity N, et al. (2012) Sustained translational repression by eIF2alpha-P mediates prion neurodegeneration. Nature 485: 507–511.
39. Cohen E, Avrahami D, Frid K, Canello T, Levy Lahad E, et al. (2013) Snord 3A: a molecular marker and modulator of prion disease progression. PLoS One 8: e54433.
40. Lesne SE, Sherman MA, Grant M, Kuskowski M, Schneider JA, et al. (2013) Brain amyloid-beta oligomers in ageing and Alzheimer's disease. Brain.
41. Santacruz K, Lewis J, Spires T, Paulson J, Kotilinek L, et al. (2005) Tau suppression in a neurodegenerative mouse model improves memory function. Science 309: 476–481.
42. Rinne JO, Brooks DJ, Rossor MN, Fox NC, Bullock R, et al. (2010) 11C-PiB PET assessment of change in fibrillar amyloid-beta load in patients with Alzheimer's disease treated with bapineuzumab: a phase 2, double-blind, placebo-controlled, ascending-dose study. Lancet Neurol 9: 363–372.