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Investigating the Role of PrP and Amyloid Beta Proteins in AD and Other Protein-Misfolding Diseases

By Steven Cornish

Abstract

Prion diseases are very rare, neurodegenerative diseases caused by misfolding of the Prion protein. The pathologies created by the misfolded protein are remarkably similar to those of Alzheimer's disease. This paper provides a comprehensive study of the genomic and proteomic similarities between Prion proteins and several proteins implicated in Alzheimer's disease (AD). While genomic similarity has been proven, studies are unclear as to whether any genomic mutations in *PRNP* affect the progression of AD. Studies have definitively proven that Prion protein interacts on a molecular level with several proteins implicated in AD. These interactions require further study to definitively prove whether they speed or slow AD progression, but current evidence indicates that Prion protein interaction will lead to faster progression of AD *in vitro*.

Introduction

The *PRNP* gene is best known for its role in Prion diseases. It produces a protein named PrP^{C} in its normal form and PrP^{Sc} in its misfolded, infectious form. The exact structural changes that result from the misfolding are unknown at this time. The misfolded PrP^{Sc} has led to the classification of new diseases known as either Prion diseases or Transmissible Spongiform Encephalopathies. These diseases are characterized by both the buildup of protein plaques in the brains of afflicted persons and

holes where nervous tissue has been degraded [Ahn 2006]. Prion diseases have been identified in sheep as Scrapie, cattle as Bovine Spongiform Encephalopathy, and Chronic Wasting Disease in deer and elk. In humans, Prion diseases include Kuru, Creutzfeldt-Jakob disease, and Fatal Familial Insomnia. All known Prion diseases are fatal, transmissible, and incurable [Bellingham, 2009].

The PrP^C protein is a GPI anchored plasma membrane protein that is abundantly expressed in the central nervous system but also appears in many other cell types. A full picture of the cellular function of PrP^C has not emerged, but it has been shown to function in intracellular copper homeostasis, as a reducer of oxidative stress, as a regulator of cytoskeletal formation, and as an antiapoptotic protein [Schmitz, 2013].

While Prion diseases are rare, neurodegenerative Alzheimer's Disease (AD) is much more common in humans. Clinical symptoms of AD include progressive memory deterioration and cognitive deficiency [Jeong, 2009]. Both Prion diseases and AD include formation of protein plaques and neurofibrillary polymers in nervous tissue, the effects of which will be fully discussed later.

The goal of this paper is to organize ideas from several research papers to gain a better understanding of how the *PRNP* gene and the PrP^C protein may affect the clinical progression of AD. After discussing *PRNP* genomics, this paper will explore PrP^c proteomics in relation to AD and protein folding mechanisms. Finally, possible future treatments for AD and Prion protein diseases will be analyzed.

Discussion

Genomics

Codon 129 in *PRNP* is may be associated with higher susceptibility to Creutzfeldt-Jakob Disease (CJD) depending on whether the amino acid methionine or valine is encoded. Methionine is implicated in higher susceptibility to CJD even if only one methionine is present [Smid 2013]. Several studies have also investigated whether PrP^C methionine or valine polymorphisms influence the incidence of AD. Homozygous valine individuals (i.e. valine at codon 129 on both chromosomes) are resistant to Prion diseases [Ahn 2006]. Researchers hypothesized that resistance to AD might also be conferred by the valine homozygosity at codon 129. In Dutch, German, and Polish studies, a strong correlation between valine homozygosity and AD resistance was found. However, Japanese, Brazilian, Korean, and Spanish studies found no correlation. The particulars of these studies will be discussed below.

A sample of Korean individuals were recruited by researchers to compare homozygosity at codon 129 to the incidence of AD. Blood samples were collected from 297 AD patients and 217 healthy control subjects. DNA was extracted from these samples and the *PRNP* gene was screened for the single nucleotide polymorphism. The researchers found no association between codon 129 polymorphisms and incidences of AD. Interestingly, the researchers found that valine was present in lower amounts in both the control and AD patients than in the European populations measured in other studies. The researchers concluded that the presence or absence of valine may contribute to the progression of AD [Ahn, 2006].

Another study in Brazil found no correlation between codon 129 polymorphisms and AD [Smid, 2013]. The researchers enlisted 99 patients with probable AD and 111 healthy control subjects in their study. After acquiring blood samples from the subjects,

the researchers monitored the polymorphism at codon 129 using high performance liquid chromatography. The researchers then analyzed the cognitive performance of the subjects. Their results showed no significant correlation between AD and homozygosity at codon 129. This study agreed with the Korean study in that homozygosity at codon 129 of the *PRNP* gene was linked to an increased risk of Creutzfeldt-Jakob Disease [Ahn, 2006]. Furthermore, they concluded that ethnic background influences whether there is an association between polymorphisms at codon 129 and AD and CJD, but the sample size was very small to be making such large claims [Smid, 2013]. This calls into question how useful this study is when trying to understand how polymorphisms in codon 129 might lead to increased risk or earlier onset of AD.

Contradictory to the previously discussed studies that showed no association between codon 129 polymorphisms and AD, a recent study using a Spanish population showed a positive association between incidence of methionine at codon 129 and incidence of AD [Moreno, 2011]. The 21 patients in the study were recruited from the cognitive disorders unit at Donostia Hospital, which isn't ideal given the small sample size and that they are all from the same hospital. The researchers followed the same procedure as the Korean study to detect polymorphisms in codon 129 and concluded that the patient's genotype had significantly earlier onset of AD if methionine was present. However, previous studies showed a positive association with the presence of valine, not methionine, at codon 129. The researchers reexamined the *PRNP* correlation with a larger sample size of 54 patients, but were unable to repeat their results. This study is interesting because the first, smaller group of patients they studied all had a single *FTD-PGRN* allele. *FTD-PGRN* has been proven to cause higher rates of dementia in those people

with the allele [Moreno, 2011]. When they didn't select for the *FTD-PGRN* allele in their larger sample size the methionine- AD correlation disappeared. It is likely that the researchers found a false positive by studying individuals with *FTD-PGRN*.

Another study from Korea examined the incidence of AD, Vascular Dementia (VD), and *PRNP* 1368, a common polymorphism in the *PRNP* gene [Jeong, 2009]. This polymorphism exists outside of the protein coding region but may still play a role in AD incidence. The researchers hypothesized that other factors outside the protein-coding region may affect the disparate results researchers are finding about the association of methionine or valine at codon 129 and AD. The main purpose of this study was to provide a positive or negative association between *PRNP* 1368 polymorphisms and AD or VD. The number of subjects was small with 152 AD participants, 192 VD participants, and 268 control participants. The researchers found that there was no correlation between the *PRNP* 1368 polymorphism and either AD or VD. There were no significant differences in genotype and allele frequencies between VD patients and controls. The researchers mention that *PRNP* 1368 polymorphisms were found in British and German populations to be associated with AD, but this association was not seen in Dutch and Korean populations. This suggests that there is some interaction between the *PRNP 1368* polymorphism and dementia, but it is not clear how it might relate to AD. It is possible that the *PRNP* 1368 polymorphism affects the expression of the *PRNP* gene, which then results in different clinical effects. It is also possible that there are other mutations, especially in the promoter region, that may have a significant effect on a person's resistance to Prion diseases or AD.

Proteomics

Proteomic similarities between Prion and AD proteins will be the next subject of this paper. Subjects to be examined include the kinetics of fibril formation, the atomic structures of the Amyloid cross beta spines, the protein folding process, and potential use of antibodies for the treatment of protein-misfolding diseases.

Amyloid Precursor Protein (APP) is a highly conserved transmembrane protein that is converted into extracellular Amyloid Beta protein (AB) upon sequential cleavage by Beta and Gamma Secretases [Balducci, 2009]. The aggregation of the AB proteins into amyloid plaques is widely believed to be the initiator of AD. APP is involved in cell adhesion, regeneration, neuroprotection, and metal homeostasis [Schmitz, 2013]. APP may interact with PrP^C, which is another way Prions may be involved with AD [Kaiser, 2012, Kudo, 2012]. When APP is converted to AB, it binds with PrP^C and creates a neurotoxic compound. Researchers hypothesized that APP and PrP^C might be related genetically and/or functionally given the highly similar functions of APP and PrP^C, as well as a high affinity interaction between them [Kaiser, 2012].

A group of Canadian researchers were curious about the interaction between PrP^C and APP and how it affected brains cells in zebrafish [Kaiser, 2012]. The *APP* gene in humans is approximately 70%-100% similar to a homologous gene in zebrafish. The researchers began by performing knockdown experiments on the *APP a*nd *PRNP* genes in zebrafish. In a knockdown experiment, a gene is either deleted from the genome or mutated in a way to render it nonfunctional. Development, cell adhesion, and neuronal growth were all inhibited in zebrafish with both the *APP* or *PRNP* genes deleted. Early apoptosis and general CNS malformations resulted. Especially of note is that knockdown of both *PRNP* and *APP* at the same time amplified these effects. When *PRNP* was

knocked down, the cellular function of APP was greatly inhibited, and vice versa. The researchers were able to rescue the double knockdown phenotype by adding a human *PRNP* or *APP* gene. The transgenic zebrafish developed normally. This suggests that the interaction between PrP^C and APP in humans is required for normal development in the same way both are required for normal development in zebrafish. The researchers were also able to show that APP and PrP^C were directly interacting with each other by performing co-immunoprecipitation. This study is very important in showing the *in-vivo* effects of APP and PrP^C interactions. It is known that plaques in AD contain both AB and PrP^C so exploring the interaction between APP, the precursor of AB, and PrP^C is very important to better understand the pathology of AD.

While mice are significantly more similar to humans than zebrafish, researchers have had a difficult time creating a mouse model that overexpresses APP due to excessive neonatal lethality. Researchers were able to encode a double mutant form of APP under control of the *PRNP* gene promoter which allowed the mice to grow old enough for experimentation and AD symptoms to appear [Chishti, 2001]. The researchers also developed an immunization against the AB peptide, which was effective in destroying plaques while it was being consistently injected, further lengthening the lifespan of the transgenic mice. It is likely that these mice will be used extensively for AD experimentation in the future to prove that the same APP/PrP^C interaction occurs in mice as well as zebrafish.

Researchers at MIT noticed that Prion diseases and AD have similar pathologies. With this in mind, they recruited GSS patients and isolated the Prion protein present in their cells. GSS is a rare, familial transmissible spongiform encephalopathy [Come,

1993]. Sequencing was then performed on the isolated protein. From analyzing the sequenced protein, a 60 amino acid section of PrP^{Sc} was found to be very similar to the C-terminal end of the AB protein which, as discussed earlier, is the primary protein found in AD plaques. The 60 amino acid section included codon 129. The researchers next analyzed the process the Prion protein undergoes to polymerize and form the plaques. The researchers found that plaque formation requires nothing more than Prion or AB protein in solution. The 60 amino acid sequence discussed above is also required for plaque formation in both Prion and AB proteins. The researchers performed preliminary kinetic analysis of plaque formation and found that it resembled protein crystallization with the individual fibrils polymerizing in a stacked beta sheet conformation. The rate of fibril formation is also greatly increased by seeding with short, preformed fibrils [Come, 1993]. The rate determining step is likely the formation of an initial fibril that PrP monomers can build off of. The researchers also found that samples homozygous for the same amino acid at codon 129 formed plaques at a faster rate than individuals heterozygous for methionine or valine at codon 129. They hypothesized that the plaque must be made of only one type of protein in order to polymerize. In order to find sequence homology, they searched AD and PrP chains for hydrophobic sequences at least 12 amino acids long, with a high tendency to form beta sheets and low tendency to form alpha helical structures. Specifically, the sequence of amino acids from 96-111 of the Prion protein is homologous to the C-terminal AB sequence. Although this paper was published in 1993, it is important in that it is one of the first papers establishing homologous regions between Prion and AB proteins. [Come, 1993].

While there have been several computer generated models of amyloid plaque structure created, it is only with more modern technology that *in vitro* samples of the plaques have been imaged. The first research on amyloid plaque structure performed with X-ray Microcrystallography occurred in 2007 and created a very accurate structural model of the basic fibril, a model that has been confirmed by other studies since then [Sawaya, 2007]. The structural model created is homologous for some 30 fibril-forming proteins, including Prion proteins. The researchers were able to improve on Come et al's [1993] research on finding the key sequence required for fibril formation. This supports the hypothesis that within each protein, there is a fibril forming sequence that is highly conserved among proteins of that class. The process of fibril formation likely begins when hydrophobic segments in several identical protein molecules are exposed, allowing them to come together and crystalize. Once this initial step is complete, it becomes much easier for future monomers to add and continue crystallization [Sawaya, 2007]. This finding agrees with those presented in another study by Come et al [1993], which showed that the initial formation of a single crystal is rate determining.

Based on the studies discussed above by Come et al [1993] and Sawaya et al [2007], researchers at the University of Toronto isolated PrP(106-126) and found that it exhibited many characteristics of the full length PrP, including fibril formation and crystallization characteristics [Walsh, 2009]. They used primarily solid-state NMR to examine the fibrils, but also performed Transmission Electron Microscopy (TEM) and Atomic Force Microscopy (AFM) measurements to further elucidate the 3D structure of the fibrils. The structure they found is in agreement with the steric zipper model proposed by Sawaya et al [2007] and resembles the quaternary packing observed for fibrils of the

beta amyloid protein. Their model also confirmed that Prion proteins cannot efficiently form fibrils without the c-terminus, confirming the findings of Come et al [1993]. Clearly, the core structure for amyloid fibrils is PrP(106-126)

Although a good model of the structure of PrP has been developed, research concerning the actual misfolding process is still in its early stages and the mechanism of misfolded Prion propagation has never been observed in real time. Just a few months prior to writing this paper, research was published in the Journal of Biological Chemistry concerning charge neutralization as a precursor step in Prion folding.

Researchers at the NAIAD, Rocky Mountain Laboratories, analyzed the Prion protein sequence and identified possible obstacles to tight beta sheet packing. They found that four closely spaced lysines, which are positively charged, in residues 101-110 would likely inhibit fibril formation. By neutralizing the positive charges, nonpathogenic Prion proteins formed fibrils similar to PrP^{SC}. During another experiment, the researchers also replaced the lysines with neutral amino acids, which also led to polymerizing behavior similar to PrP^{SC}. In both experiments, the synthetically produced Prion protein the researchers used never gained infectivity, so there must be another factor that is missing in conversion of PrP^{C} to PrP^{SC}. The researchers also tried deleting all the lysines in that area and found that it inhibits fibril formation [Groveman, 2014].

In further research performed at the NAIAD, transgenic mice without the GPI anchor sequence of PrP were created. The GPI anchor is found on the C-terminal end of the protein. They infected the GPI- and wild-type (WT) mice with PrP^{SC} and observed the infectivity and presence of amyloid plaques at various time intervals. They found that GPI- mice had consistently fewer amyloid plaques than WT mice, but there continued to

be neuropathological lesions in both. GPI- mice were less symptomatic than WT mice throughout the entire disease process, likely due to the decreased ability of Prion protein to form plaques without the C-terminal sequence. Infection by PrP^{SC} still resulted in infection and classical Amyloid disease symptoms, though they are less severe in GPI- mice compared to WT mice. Therefore, formation of Amyloid plaques is not vital to the disease process, but it does measurably affect it [Chesebro, 2005].

Researchers at the University of London and University of Bath hypothesized that PrP(95-113) would bind to AB proteins. As we discussed earlier, there seems to be a highly conserved amino acid sequence that is required for fibril formation. The experiment involved mixing purified Prion protein with AB oligomers, and separately with preformed AB tendrils. The researchers found that a very small amount of Prion protein will strongly inhibit amyloid fibril formation, as well as binding preformed AB tendrils and converting them back to oligomeric form [Younan 2013]. Destruction of AB tendrils might sound like a positive effect, but some studies have shown that AB oligomers are significantly more toxic than AB fibrils [Westaway 2012]. If oligomeric AB is truly more damaging, Prion protein may speed up the progression of AD.

While it has been proven that Prion protein and AB interact on the molecular level, proving that this interaction is required for toxicity makes it much more relevant for study. Kudo et al [2012] proved that the PrP^{C} protein is necessary for AB-induced neuronal cell death. They began by treating brain cultures from WT mice with AB to induce cell death. When this study was repeated on *PRNP*-/- mice, the number of dead cells dramatically decreased. Another experiment was performed using an antibody that binds to PrP^{C} and prevents its binding to AB. When AB and the PrP^{C} antibody were

added to the cultures with PrP^C, they found that the number of dead cells was greatly decreased compared to the control. Mutations in the region of PrP^C that binds AB also resulted in a decrease in cell death. Furthermore, injection of AB protein into the brains of both WT and *PRNP* -/- mice led to a reduction in cell death [Kudo, 2012].

In another study looking at AB and PrP^C interactions, researchers concentrated on the impairment of synaptic plasticity and memory caused by the addition of AB to the brains of WT and *PRNP* -/- mice [Balducci, 2010]. The researchers first showed that AB proteins induce reversible memory impairment that is preventable by treatment with an anti-AB antibody. The researchers also found that memory impairment could be reversed by stopping injections of AB protein. Therefore, destroying AB in humans might reduce symptoms of AD. Continuing on, the researchers validated the interaction between AB and PrP^C. They found, in agreement with the previous study, that they are tightly bound together and certainly interact in the brain. When comparing the memory impairment of *PRNP* -/- and *PRNP* +/+ mice that had AB injected, the researchers found no difference. Therefore, it would appear that while the PrP^C and AB interaction is important for cellular death, AB does not need PrP^C to bring the other AD pathologies.

Of further interest in the exploration of the PrP^C and AB interaction is how PrP^C affects APP cleavage to AB. An effect that PrP^C has on AB expression is that when PrP^C is overexpressed, expression of AB is noticeably decreased. An interaction between APP and PrP^C may be responsible for the decreased cleavage of APP to AB. Expression of the two secretases that perform the cleavage of APP to AB remained the same.

Increased levels of PrP^C also decrease AD pathology caused by Tau proteins depositing into fibrils [Schmitz, 2013]. Fibrils consisting of hyperphosphorylated Tau

proteins form neurofibrillary tangles. These tangles are hallmarks of AD pathology. Tau is normally involved in tubulin polymerization, but when it is hyperphosphorylated it loses functionality. PrP^C was able to down regulate expression of Tau and several of its related isoforms when it was overexpressed in the neuronal cells. These results support the hypothesis that PrP^C is involved in the reduction of cellular stress and cellular death that results from AB aggregates and Tau fibril creation. The exact mechanism still isn't clear, but further studies may better reveal it.

The pathology involved with AB and PrP^C interacting with the Src kinase Fyn, which is involved in Tau hyperphosphorylation and AB/Tau toxicity, may also be implicated in AD pathology [Larson, 2012]. The researchers found that Fyn and PrP^C levels correlate strongly with the incidence of AD. They then tested whether PrP^C and Fyn interacted with each other, or just happened to have increased expression at the same time. Their examination showed a direct interaction between PrP^C and Fyn, as well as between PrP^C and AB. These findings are in agreement with the earlier studies discussed in this review. This study is very helpful in more fully describing the mechanism by which PrP^C is able to down regulate Tau by binding to Fyn and stopping its ability to phosphorylate Tau.

A study performed at the University of Leicester by Halliday et al explored the possibility that the spread of neurodegenerative diseases through Prion misfolding is a generic, conserved process and occurs in both AD and Prion diseases. The researchers found that Tau, AB, and alpha-Synuclein are all capable of the templated conformational change that was initially discovered by study of Prion diseases. By templated conformational change, the researchers mean that once one protein becomes misfolded, it

will form a template that nonmisfolded proteins will interact with and misfold. The researchers decided to examine whether it was the misfolding itself that caused the neurotoxicity, or if it is an interaction between the misfolded protein and another molecule that creates the toxicity. The researchers found that PrP^{SC} likely causes neurotoxicity by activating the Unfolded Protein Response (UPR), which is a response by the cell to decrease protein synthesis overall, while increasing protein chaperone synthesis to enhance correct protein folding. Rising levels of PrP^{SC} activates this response as the body will recognize PrP^{SC} as a misfolded protein. The researchers gave mice infected with PrP^{SC} a drug to stop the UPR response, which resulted in lessened symptoms and reduced pathogenesis. Stopping the UPR appears to actually be neuroprotective; however, the drug used was extremely toxic and had deleterious side effects. Deactivating the UPR in AD might be similarly neuroprotective [Halliday 2014].

While some extensive research has been concerned with the residues most similar to AB proteins, other parts of the PrP amino acid sequence haven't been researched in as much detail. One of those sequences that has been studied in detail is PrP(185-206), as it is homologous to the hydrophilic segment 1-28 of AB peptide. Uzbekistani scientists found that the sequence in question interacts strongly with negatively charged planar lipid membranes by making the phospholipid bilayers permeable. It introduces multiple structural deficits, increasing membrane permeability [Sonkina 2010]. However, no well-defined channels are formed. Due to the structural changes PrP causes in membranes, it is possible that part of the neuropathology of AD or PrP is due to these changes.

Given the complex and possibly damaging effects the Prion protein has, it has been theorized that it might be better to completely remove *PRNP* from the genome.

However, when researchers made mice that overexpressed APP without any copies of *PRNP*, 90% of those mice died by 100 days. Mice that were APP overexpressing with both copies of PRNP had 40-60% mortality. PrP must have a neuroprotective effect in decreasing the amount of APP present [Westaway 2012].

As present research indicates completely removing PrP from the genome is extremely deleterious to life expectancy, a group of researchers instead developed an antibody just one year ago that is specific for Prion and AD proteins. The anti-Prion antibody was able to cross an artificial blood brain barrier and permanently ended Prion replication in cells with infectious PrP^{SC}. With ELISA and immunohistochemistry, they proved that anti AB and Tau antibodies were binding their targets [David 2014]. This research seems to be the most promising route to take in treating AB and Prion diseases, as both diseases begin with "good" proteins, which are changed into "bad" proteins by misfolding and aggregation.

Conclusions

The interactions between the *PRNP* gene and its protein product PrP^{C} with other factors involved in AD are truly fascinating. While the connection between mutations in *PRNP* and susceptibility or severity of AD is tenuous, there is certainly a strong proteomic relationship between PrP^{C}, APP, and AB. Codon 129 has been of specific interest, but the studies are not in agreement on whether there is any correlation between mutations in the codon and AD. Explorations of mutations outside the coding region are also inconclusive as to whether or not they affect AD.

The protein PrP^{C} has been definitively shown to interact with APP, AB, and Tau proteins, all of which are involved in the pathology of AD. The studies universally

showed a strong interaction between PrP^C and APP/AB in the progression of AD. Cell death can only occur in the presence of PrP^C , which has also been shown to down regulate Tau. Tau is a component in the characteristic protein fibrils that form in the brains of people with AD. The mechanism of down regulation may involve the Src kinase Fyn, which hyperphosphorylates the Tau protein and leads to fibril formation.

Advances in modern technologies have only recently made study of specific sequences in the PrP/AB proteins possible. On a proteomic level, researchers have been able to identify several homologous sequences of interest in the PrP protein. The functions of those sequences have also been investigated, the most relevant of which are membrane permeability increases and the sequence required for fibril formation. Researchers have also been able to extensively study the structure of both PrP and AB protein fibrils while also analyzing crystallization characteristics and rates of formation.

PrP, AB, and Tau antibodies have been shown to bind with their respective proteins with high specificity and permanently stop misfolded proteins from aggregating or reproducing. Previous treatment methods have concentrated on genetic manipulation, but have been mainly ineffective.

Future Directions

Since the first discovery of Prion diseases and the Prion gene *PRNP*, researchers have been studying the effects of PrP^C and its mutations on bodily homeostasis. In order to gain a better understanding of how *PRNP* is related to AD, studies of a larger sample size and from more countries would be very helpful. It is also very interesting how the results of the studies tend to correlate with ethnic backgrounds, so figuring out why that happens is a viable goal of further studies. At the protein level, PrP^C interacts with

numerous other proteins and enzymes that are not all well characterized. The majority of studies in this paper are from the last few years, so there is not a strong foundation of literature to base claims on. Of special interest are the regulatory interactions PrP^{C} has, including the APP to AB change. The number of studies in this area is still limited, but there may be other proteins PrP^{C} interacts with.

Of special note is that while fibril structure has been studied in some detail, all theories of the structure of PrP^{C}/PrP^{Sc} are based solely on computer models. Future studies should address this gap in knowledge. In addition, the method of infectivity and protein folding process that PrP undergoes is poorly understood. No research studies have been able to observe the misfolding mechanism in real time. How PrP^{SC} passes on its infectivity to other proteins is mere hypothesis.

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