FUNDAMENTAL NEUROSCIENCE
Zelić DF. The prion protein and Creutzfeldt-Jakob disease. pp. 323 – 328

THE PRION PROTEIN AND CREUTZFELDT-JAKOB DISEASE

Dora Franka Zelić
University of Zagreb, School of Medicine
0000-0001-9418-8996

Abstract

The prion protein, which stands for *proteinaceous infectious particle*, has mystified scientists for years. To date we don't fully understand its physiological role so in this article we'll be focusing on the pathology. The prion protein causes several diseases in humans: Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträussler-Scheinker syndrome (GSS), fatal familial insomnia (FFI), and kuru. They are also called transmissible spongiform encephalopathies (TSEs). Prions don't cause diseases only in humans - in animals they cause scrapie in sheep and goats, bovine spongiform encephalopathy (BSE) or mad cow disease, and several other encephalopathies. These diseases share a lot of similarities as they are incurable, uniformly fatal, have long incubation times, and cause spongiform vacuolation, neuronal cell loss, astrocytosis, and accumulation of amyloid plaques in the central nervous system (CNS). Signs and symptoms can vary from psychiatric to neurological disorders. Here we will focus on CJD, as it is the most common TSE. Diagnosis of CJD *premortem* is still a problem for clinicians in part due to the rarity of the condition, similarity to some other neurodegenerative diseases, and lack of specific diagnostic procedures. Current World Health Organisation (WHO) diagnostic criteria date back to 1998 though the Center for Disease Control (CDC) offers newer guidelines published in 2010. Since CJD is incurable, early diagnosis would allow patients and their families to prepare for the expected outcome and possibly help cope with the loss of a loved one.

KEYWORDS: Creutzfeldt-Jakob disease, neurodegeneration, prion, prion disease, transmissible spongiform encephalopathies

INTRODUCTION

Prions (prion proteins or PrP) are infectious particles built entirely out of proteins. The word itself is an abbreviation of *proteinaceous infectious particle*. PrP exists in different isoforms which define its behaviour.[1] For instance, cell prion protein (PrPC) exists as a membrane protein in our cells and its role is still being investigated, but PrP found in organisms with prion diseases, such as in scrapie (PrPSc), is the cause of these diseases. Human prion diseases, or transmissible spongiform encephalopathies (TSEs), make an interesting group for they can be sporadic, acquired or genetic and are uniformly fatal.[2] This article will focus on the most common one, Creutzfeldt-Jakob disease (CJD). Among other TSEs there is kuru, which is sporadic, as well as fatal familial insomnia (FFI) and Gerstmann-Sträussler-Scheinker syndrome (GSS), which are both genetic.[2]

CJD is a fatal progressive neurodegenerative disorder which presents with mostly psychiatric and neurological symptoms accompanied by classical histological findings: spongiform change, neuron loss, and astrogliosis.[3] It annually affects one to two persons per 1,000,000 worldwide.[4] Based on how the disease was acquired we can divide it into 4 types: sporadic CJD (sCJD) caused by a somatic mutation in the PRNP gene or a spontaneous conformational change in the PrP, familial CJD (fCJD) which is inherited, iatrogenic CJD (iCJD) from various human-to-human implants, and the most recently identified type variant CJD (vCJD) acquired by the transmission of bovine spongiform encephalopathy (BSE) (Table 1).[2]

THE PRION PROTEIN

PrPC is a glycosylated, glycosyl phosphatidyl inositol (GPI) anchored protein of 209 amino acids and a mostly alpha-helical structure (40%).[5,6] It can be found in 'membrane rafts' rich in cholesterol and sphingolipids. The gene which encodes PrPC, called the prion protein gene (PRNP), is located on human chromosome 20. Normal PRNP shows genetic polymorphism at codon 129, where methionine (M) or valine (V) may be encoded.[6] PrPC is highly conserved across species which allows prion diseases to be transmitted between species.[7]

Features	sCJD	vCJD	fCJD
Mean age of onset	60-70 years	28 years	60 years
Duration of illness	5 months	14 months	6 months
Predominant clinical features	Rapid cognitive decline, myoclonus	Early psychiatric symptoms, then cognitive decline	Similar to sCJD
MRI findings	60-70% have hyperintensity in basal ganglia or cortex	Pulvinar sign in 90%	Basal ganglia and cortical hyperintensity
EEG findings	60-70% show PSWCs	PSWCs negative	75% show PSWCs
14-3-3 status	90% positive	50% positive	Similar to sCJD
Genetics	70% MM1	100% MM	*PRNP* mutation

Table 1. Findings in different types of CJD. iCJD is extremely similar to sCJD so it won't be described separately.
Adapted from: Manix M, Kalakoti P, Henry M, et al. Creutzfeldt-Jakob disease: updated diagnostic criteria, treatment algorithm, and the utility of brain biopsy. Neurosurg Focus. 2015;39(5):E2. doi:10.3171/2015.8.FOCUS15328.

The protein contains a single bisulfide bridge, two N-glycosylation sites, as well as a C-terminal globular domain.[5] The physiological role of PrPC is still being examined but some theories have emerged (Table 2). The N-terminal region of the protein contains octapeptide repeat motifs which have five Cu2+ binding sites.[8] The protein's expression alters copper uptake into cells. Therefore, it has been suggested that PrPC plays a role in copper metabolism. It may even affect our cells capacity to fight oxidative stress through superoxide dismutase (SOD), an enzyme which neutralises superoxide radical (O2-), whose two out of three human forms require copper to properly function.[9] Another proposed role is that PrPC acts as a scaffold protein meaning that it binds several members of a signalling pathway optimizing downstream signal transfer, for instance by regulating the activity of phosphatidylinositol 3-kinase (PI 3-kinase), which is an enzyme that's part of an intracellular signalling pathway important in regulating the cell cycle.10 Some researchers even speculate that PrPC may play a role in maintaining long-term memory.11 So what makes PrPSc so different from PrPC? Although they have the same primary structure, PrPSc has a 45% beta-sheet structure (Table 3).[6] This is enough to make it resistant to degradation by proteases and highly insoluble.12 If PrPC is structurally altered and converted into a proteinase K-resistant form *in vitro* it's called protease-resistant PrPSc-like protein (PrPres). How can PrPSc replicate if it's made only of amino acids? PrPSc converts normal PrPC molecules into it's own misfolded form. The conversion process is not yet fully understood but PrPSc seems to serve as a template and autocatalyses the process which can occur in a cell-free system.[2] Accumulated PrPSc then forms amyloid aggregates which can be found histologically.[6]

CREUTZFELDT-JAKOB DISEASE

CJD was first described in the 1920s by two German physicians, Hans Gerhard Creutzfeldt and Alfons Maria Jakob, after whom the disease was named.[6,13] For a long time it was speculated that a virus causes the disease and to this day there are many theories, but the protein only theory which states that PrPSc causes the disease, is mostly accepted. Marked neuronal loss, spongiform change and astroglyosis, are typically found in CJD upon histopathological examination. Spongiform change is the most specific and presents with a fine vacuolation, mostly in dendrites, of the grey matter with round or oval vacuoles containing curled membrane fragments and amorphous material. Neuronal loss seems to mostly affect GABAergic neurons and usually spares the hippocampus and dentate gyrus, which are considered to be most vulnerable. Clinical presentation differs between each type and subtype of CJD but typically we can see a progressive encephalopathic illness with dementia, cerebellar ataxia and myoclonus.[3] General diagnostic criteria for CJD by the World Health Organisation (WHO) are shown in Table 4 and the Center for Disease Control (CDC) guidelines are in Table 5. We will now discuss each type in more detail.

SPORADIC CJD

Sporadic CJD (sCJD) is the most common form of the disease with a prevalence of 85%.6 The etiology is probably a somatic mutation in the *PRNP* gene or a random structural change in the PrPC causing it to change into PrPSc. It affects mostly the middle-aged and elderly with a median survival

FUNDAMENTAL NEUROSCIENCE
Zelić DF. The prion protein and Creutzfeldt-Jakob disease. pp. 323 – 328

Level	Process
Molecular	Homeostasis of copper
	Ion flux
	Transport of metabolites
	Redox homeostasis
Cellular	Cell proliferation
	Cell adhesion
	Cell differentiation
	Cell survival
	Cell death
	Neurite overgrowth
	Myelin maintenance
	Synaptic transmission
	Synaptogenesis
	β-amyloid toxicity
	T-cell activation
System	Memory
	Sleep
	Embryogenesis
	Inflammation
	Stem cell renewal
	Muscle physiology
	Glucose homeostasis

Table 2. Proposed physiological roles of the prion protein.
Source: Linden R. The Biological Function of the Prion Protein: A Cell Surface Scaffold of Signaling Modules. Front Mol Neurosci. 2017;10:77. doi:10.3389/fnmol.2017.00077.

of around 4 months from the onset of the disease. sCJD is divided into six subtypes based on the two types of human PrPSc and on the polymorphism of codon 129 of the *PRNP* gene. Type 1 prion has a relative molecular mass of 21kDa and Type 2 of 19kDa.[3] The most common sCJD subtype is MM1 which presents with cognitive impairment, myoclonus, cerebellar ataxia and psychiatric symptoms.[6] There are also some clinical variants such as the Heidenhain variant where the patient presents with diplopia, blurred vision, cortical blindness and/or visual hallucinations within the first week of illness, or the Oppenheimer-Brownell variant in which there are no cognitive or visual impairments in the first week, only ataxia. The Heidenhain variant is found only in the MM1 subtype.14 Periodic sharp wave complexes (PSWCs) are an EEG finding specific for sCJD and can be observed in most patients during the duration of the illness, with the exception of the VV1 subtype.[6,15] Cerebrospinal fluid (CSF) can be tested for 14-3-3 protein, which appears after neuronal destruction and is positive in almost all patients and in all types of sCJD.[6] This biomarker, unlike PSWCs, is not specific to sCJD and can be found in other conditions where neurons are being destroyed.[6]

IATROGENIC CJD

Iatrogenic CJD (iCJD) has been linked to intracerebral electrodes, corneal transplantations, dura mater grafts, and growth hormone injections.[6,16,17] The incubation period reflects the site of inoculation; for instance, the incubation time when contaminated electrodes were placed directly into the brain was 16-28 months, whereas peripheral growth hormone injections from contaminated cadavers took 5-30 years to bring about iCJD.[6] There are cases of probable vCJD transmission via blood transfusions.[18] This alarmed the Food and Drugs Administration (FDA) which banned blood donation from people who have spent 6 months or more in the UK between 1980 and 1996.[19] Clinically it resembles sCJD with a different age of onset depending on the age at which the patient received a contaminated transplant or implant.[6]

FAMILIAL CJD

Familial CJD (fCJD) is caused by an inherited mutation in the *PRNP* gene which encodes the prion protein. Since there are many different mutations that can occur, there are also many presentations of the disease but it mostly resembles sCJD. It's transmitted in an autosomal dominant pattern meaning that first-degree relatives have a 50% chance of inheriting the disease.[20] Two other diseases are caused by a mutation in the *PRNP* gene; Gerstmann-Sträussler-Scheinker (GSS) syndrome and fatal familial insomnia (FFI). GSS is distinguished histologically by widespread, large, multicentric amyloid plaques within the cerebral cortex.[21] FFI is caused by a mutation at position 178 (D178N) accompanied by a methionine at codon 129 of the mutated allele, whereas fCJD is accompanied by a valine at the same codon on the mutated allele.[22] Age of onset differs but for fCJD it's around 60 years of age.[6]

PrP^{C}	PrP^{Sc}
Non-infectious	Infectious
Digested by proteinase K	Resistent to proteinase K
40% alpha-helix	45% beta-sheets
Soluble	Insoluble
Monomeric	Multimeric

Table 3. Differences between PrPC and PrPSc.
Based on references 6 and 8.

Probable CJD	Possible CJD
Progressive dementia; **and** at least two out of the following four clinical features:	
1. Myoclonus 2. Visual or cerebellar disturbance 3. Pyramidal/extrapyramidal dysfunction 4. Akinetic mutism; **and**	
1. A typical EEG during an illness of any duration, **and/or** 2. A positive 14-3-3 CSF assay and a clinical duration to death <2 years; 3. Routine investigations should not suggest an alternative diagnosis.	1. No EEG or atypical EEG; **and** 2. Duration <2 years

Table 4. WHO diagnostic criteria for CJD from 1998.
Source: Global surveillance, diagnosis and therapy of human Transmissible Spongiform Encephalopathies: Report of a WHO consultation. Geneva, Switzerland, February 9-11, 1998. https://www.who.int/csr/resources/publications/bse/whoemczdi989.pdf. Accessed May 1, 2017.

Definite CJD	Probable CJD	Possible CJD
Diagnosed by: 1. Standard neuropathological techniques, **and/or** 2. Immunocytochemically, **and/or** 3. Western blot confirmed protease resistant PrP, **and/or** 4. Presence of scrapie-associated fibrils	**Progressive dementia**; **and** at least two out of the following four clinical features: 1. Myoclonus 2. Visual or cerebellar disturbance 3. Pyramidal/extrapyramidal dysfunction 4. Akinetic mutism;	
	and a positive result on at least one of the following laboratory tests: a) PSWCs on EEG b) Positive 14-3-3 protein in the CSF with a disease duration of < 2 years c) High signal abnormalities in caudate nucleus and/or putamen on	**and** the absence of a positive result for any of the three laboratory tests that would classify a case as "probable" **and** disease duration of < 2 years **and** without routine investigations indicating an alternative diagnosis.

Table 5. CDC diagnostic criteria for sCJD from 2010.
Source: Diagnostic Criteria | Creutzfeldt-Jakob Disease, Classic (CJD) | Prion Disease | CDC. https://www.cdc.gov/prions/cjd/diagnostic-criteria.html. Updated February 11, 2015. Accessed May 1, 2017.

VARIANT CJD

Variant CJD was first described in the UK in the 1990s following a BSE epidemic.[23] People were infected by eating food contaminated with BSE. It differs from other types of CJD by a younger mean age of onset, 28 years, and a longer period of illness duration of 14 months.[3] The incubation period is about 11-12 years. At the beginning of the disease, symptoms are mostly psychiatric with more classical CJD symptoms, such as ataxia, appearing later on.6 EEG doesn't show PSWCs and CSF 14-3-3 is positive in less than 50% of patients.[3] Spongiform change with gliosis is most prominent in the basal ganglia and cerebellum, especially in the pulvinar.[6] This change is consistent with the characteristic hyperintensity in the posterior thalamus shown on an MRI which is thus called the 'Pulvinar Sign'. The sign is positive in 90% of cases.[3] PrPSc forms florid plaques and accumulates throughout the cerebral cortex, but even more intriguing is the accumulation of PrPSc in the lymphoreticular system which is only seen in vCJD. After a person eats a prion contaminated product, PrPSc is transported through the intestinal epithelium by M-cells to Peyer's patches. There, PrPSc accumulates within dendritic cells and disseminates via the blood stream to the rest of the lymphoreticular system. Autonomic nerves or lymphoid tissue carry the PrPSc to the central nervous system.[2] To date, all vCJD cases have been in individuals with methionine homozygosity at codon 129 of *PRNP*.[6]

TREATMENT

Many drugs, such as antiviral medications, amphotericin B, flupirtine, quinacrine, pentosan polysulphate, doxycycline and more, have been tested for treating CJD but there are none that stop or slow down the progression of the disease to this day.[24,25] The search for a cure continues, but until it's found we can only hope to alleviate pain and make patients as comfortable as possible.

FUNDAMENTAL NEUROSCIENCE
Zelić DF. The prion protein and Creutzfeldt-Jakob disease. pp. 323 – 328

CONCLUSION

CJD includes a very wide set of clinical manifestations which vary in duration and age of onset in different types. For this and many other reasons, such as a lack of definite diagnostic tests and similarity to some other neurodegenerative diseases (Alzheimer's disease, Lewy body dementia, frontotemporal dementia…), it usually passes unrecognized during the patient's life. Although there is no cure for CJD so far, correct diagnosis is important to help patients and their families prepare for the disease outcome. Improving the patient's life and the lives of those around them should be a priority. There is yet much to be discovered regarding the prion protein, both in PrPC and PrPSc form, and hopefully one day we'll get closer to changing the fatal outcome of CJD.

REFERENCES:

1. Pan KM, Baldwin M, Nguyen J, et al. Conversion of alpha-helices into beta-sheets features in the formation of the scrapie prion proteins. *Proc Natl Acad Sci*. 1993;90(23):10962-10966. doi:10.1073/pnas.90.23.10962.
2. Hilton DA. Pathogenesis and prevalence of variant Creutzfeldt-Jakob disease. *J Pathol*. 2006;208(2):134-141. doi:10.1002/path.1880.
3. Sikorska B, Knight R, Ironside JW, Liberski PP. Creutzfeldt-Jakob disease. In: Ahmad SI, ed. *Neurodegenerative Diseases*. 1st ed. Springer US; 2012:76-90. doi:10.1007/978-1-4614-0653-2.
4. Kojima G, Tatsuno BK, Inaba M, Velligas S, Masaki K, Liow KK. Creutzfeldt-Jakob disease: a case report and differential diagnoses. *Hawaii J Med Public Health*. 2013;72(4):136-139. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3689509&tool=pmcentrez&rendertype=abstract.
5. Linden R, Cordeiro Y, Lima LMTR. Allosteric function and dysfunction of the prion protein. *Cell Mol Life Sci*. 2012;69(7):1105-1124. doi:10.1007/s00018-011-0847-7.
6. Manix M, Kalakoti P, Henry M, et al. Creutzfeldt-Jakob disease: updated diagnostic criteria, treatment algorithm, and the utility of brain biopsy. *Neurosurg Focus*. 2015;39(5):E2. doi:10.3171/2015.8.FOCUS15328.
7. Martins VR, Brentani RR. The biology of the cellular prion protein. *Neurochem Int*. 2002;41(5):353-355. doi:10.1016/S0197-0186(02)00054-2.
8. Zomosa-Signoret V, Arnaud J-D, Fontes P, Alvarez-Martinez M-T, Liautard J-P. Physiological role of the cellular prion protein. *Vet Res*. 2008;39(4):9. doi:10.1051/vetres:2007048.
9. Fukai T, Ushio-Fukai M. Superoxide Dismutases: Role in Redox Signaling, Vascular Function, and Diseases. *Antioxid Redox Signal*. 2011;15(6):1583-1606. doi:10.1089/ars.2011.3999.
10. Linden R. The Biological Function of the Prion Protein: A Cell Surface Scaffold of Signaling Modules. *Front Mol Neurosci*. 2017;10:77. doi:10.3389/fnmol.2017.00077.
11. Shorter J, Lindquist S. Prions as adaptive conduits of memory and inheritance. *Nat Rev Genet*. 2005;6(6):435-450. doi:10.1038/nrg1616.
12. Trevitt CR, Singh PN. Variant Creutzfeldt-Jakob disease : pathology , epidemiology , and public health implications 1 – 4. 2003;1986(3):3-8.
13. Jakob A. Uber eigenartige Erkrankungen des Zentralnervensystems mit bemerkenswertem anatomischen Befunde. 1921.
14. Appleby BS, Appleby KK, Crain BJ, Onyike CU, Wallin MT, Rabins P V. Characteristics of established and proposed sporadic Creutzfeldt-Jakob disease variants. *Arch Neurol*. 2009;66(2):208-215. doi:10.1001/archneurol.2008.533.
15. Wang P-S, Wu Y-T, Hung C-I, Kwan S-Y, Teng S, Soong B-W. Early detection of periodic sharp wave complexes on EEG by independent component analysis in patients with Creutzfeldt-Jakob disease. *J Clin Neurophysiol*. 2008;25(1):25-31. doi:10.1097/WNP.0b013e318163a7d5.
16. Duffy P, Wolf J, Collins G, DeVoe AG, Streeten B, Cowen D. Possible person-to-person transmission of Creutzfeldt-Jakob disease. 1974.
17. Brown P, Preece M, Brandel JP, et al. Iatrogenic Creutzfeldt-Jakob disease at the millennium. *Neurology*. 2000;55(8):1075-1081. doi:10.1212/WNL.56.7.987.
18. Llewelyn CA, Hewitt PE, Knight RSG, et al. Possible transmission of variant Creutzfeldt-Jakob disease by blood transfusion. *Lancet*. 2004;363(9407):417-421. doi:10.1016/S0140-6736(04)15486-X.
19. Questions and Answers on " Guidance for Industry : Revised Preventive Measures to Reduce the Possible Risk of Transmission of Creutzfeldt-Jakob Disease (CJD) and Variant Creutzfeldt-Jakob Disease (vCJD) by Blood and Blood Products ." https://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Blood/ucm074100.htm. Accessed April 30, 2017.
20. Clift K, Guthrie K, Klee EW, et al. Familial Creutzfeldt-Jakob Disease: Case report and role of genetic counseling in post mortem testing. *Prion*. 2016;10(6):502-506. doi:10.1080/19336896.2016.1254858.
21. Collins S, McLean CA, Masters CL. Gerstmann-Straussler-Scheinker syndrome,fatal familial insomnia, and kuru: a review of these less common human transmissible spongiform encephalopathies. *J Clin Neurosci*. 2001;8(5):387-397. doi:10.1054/jocn.2001.0919.
22. Llorens F, Zarranz J-J, Fischer A, Zerr I, Ferrer I. Fatal Familial Insomnia: Clinical Aspects and Molecular Alterations. *Curr Neurol Neurosci Rep*. 2017;17(4):30. doi:10.1007/s11910-017-0743-0.

23. Will R., Ironside J., Zeidler M, et al. A new variant of Creutzfeldt-Jakob disease in the UK. Lancet. 1996;347(9006):921-925. doi:10.1016/S0140-6736(96)91412-9.
24. Appleby BS, Lyketsos CG. Rapidly progressive dementias and the treatment of human prion diseases. *Expert Opin Pharmacother.* 2011;12(1):1-12. doi:10.1517/14656566.2010.514903.
25. Damkier HH, Brown PD, Praetorius J. Cerebrospinal fluid secretion by the choroid plexus. *Physiol Rev.* 2013;93(4):1847-1892. doi:Doi 10.1152/Physrev.00004.2013.

PRIONSKI PROTEIN I CREUTZFELDT-JAKOBOVA BOLEST

Sažetak

Prioni, zarazne proteinske čestice, već godinama zbunjuju znanstvenike. Do danas ne znamo točnu fiziološku ulogu priona pa ćemo se koncentrirati više na patologije koje uzrokuju. U ljudi uzrokuju: Creutzfeldt-Jakobovu bolest (engl. *Creutzfeldt-Jakob disease*, CJD), Gerstmann-Sträussler-Scheinkerov sindrom, smrtonosnu obiteljsku nesanicu i kuru. Zajedno ih nazivamo transmisibilnim spongiformnim encefalopatijama. Prioni ne uzrokuju bolesti samo u ljudi, već i u nekih životinja kao što su ovce, koze u kojih uzrokuje grebež (engl. *scrapie*), goveđu spongiformnu bolest ili kravlje ludilo i nekoliko drugih encefalopatija. Nabrojene bolesti dijele dosta sličnosti: neizlječive su, smrtonosne, imaju dug period inkubacije i uzrokuju spongiformnu promjenu, gubitak neurona, astrocitozu i akumulaciju amiloidnih plakova u središnjem živčanom sustavu. Simptomi su psihijatrijske i neurološke naravi. U ovom članku usredotočit ćemo se na CJD kao najčešću spongiformnu encefalopatiju. Dijagnosticiranje CJD-a prije smrti problem je s kojim se kliničari susreću zbog malog broja takvih slučajeva, sličnosti sa drugim neurodegenerativnim bolestima i nedostatka specifičnog dijagnostičkog testa. Trenutne dijagnostičke smjernice Svjetske zdravstvene organizacije su iz 1998. Američki Centar za kontrolu bolesti nudi nešto novije smjernice iz 2010. Pošto je CJD neizlječiva bolest, točna rana dijagnoza omogućila bi pacijentima i njihovim obiteljima više vremena da se pripreme za očekivani ishod te bi se bolje nosili s bolešću.

KLJUČNE RIJEČI: Creutzfeldt-Jakobova bolest, neurodegeneracija, prijenosne spongiformne encefalopatije, prioni, prionske bolesti

Received May 1, 2017.
Accepted September 29, 2017