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Prion Epidemiology And The Npdpsc's Experience With Rt-Quic

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ABSTRACT

Prion diseases, or transmissible spongiform encephalopathies, constitute a wide array of invariably fatal rapidly progressive neurodegenerative illnesses that affect both humans and animals. The gold standard of diagnosis for these diseases is through neuropathologic examination of brain tissue following autopsy. In September 2018, the Centers for Disease Control and Prevention (CDC) added positive real-time quaking-induced conversion (RT-QuIC) test result as a likely indicator of certain human prion diseases. With high sensitivity and specificity approaching 100%, RT-QuIC has quickly become one of the most powerful antemortem diagnostic tools.

This paper will demonstrate why changes in diagnostic criteria and reporting metrics are appropriate and innovative in the diagnosis and surveillance of prion disease. An introduction to prion biology and epidemiology in the 21st century is followed by the presentation of the National Prion Disease Pathology Surveillance Center's (NPDPSC) experience with 2nd generation RT-QuIC over a 3-year period. In this observational study, 10,498 unique cerebrospinal fluid (CSF) samples taken from suspected cases of prion disease were sent to the NPDPSC. 567 of these cases also went on to autopsy; autopsy results were then used to determine RT-QuIC's sensitivity (90.3%) and specificity (99.8%).

Type of prion disease, illness duration, and various demographic characteristics were analyzed to determine possible influences on RT-QuIC results. Sensitivity was found to be lower among rarer prion diseases, such as genetic and atypical sporadic diseases. Poor sample quality was also associated with lower sensitivity. Sporadic Creutzfeldt-Jakob Disease (sCJD) cases were more likely to produce false negative RT-QuIC results if samples were from younger individuals or from cases with longer disease durations.

In conclusion, RT-QuIC is a highly sensitive and specific test that can be an aid in ascertaining an extremely rare disease. However, this study has shown that its sensitivity and specificity can be affected by disease type, specimen quality, and demographic characteristics among individuals with suspected cases of prion disease. Moving forward, this novel assay will become an invaluable objective tool in diagnosing prion disease antemortem.

ACKNOWLEDGEMENTS

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EPIDEMIOLOGY

Biology

Uniformly rare and invariably fatal, prion diseases constitute an array of illnesses in humans, including genetic, sporadic, and acquired diseases. Although each disease differs in its clinical manifestations, these transmissible spongiform encephalopathies are most commonly characterized by rapidly progressive neurodegeneration and are all caused by the misfolding of the prion protein.

This protein (PrP) is found in abundance in the natural world including animals and humans, and in its normal, non-disease state is referred to as PrP^C. The exact function that PrP^C plays is yet to be conclusively determined, although research conducted *in vitro* and with yeast, mice, and zebrafish posit that PrP^C may have a role in cell adhesion,[1] anti-apoptotic activities, cellular copper uptake, or synaptic formation and maintenance.[2] Among genetic cases of prion disease, mutations in the *PRNP* gene, which codes for PrP^C, can cause the protein to take on an abnormal shape and transform into the disease causing prion protein (PrP^{Sc}). Acquired prion disease is caused by exposure to PrP^{Sc} from an external source such as through the ingestion of infected beef, as happened during the mad cow disease epidemic of the late 20th century. More commonly, this misfolding occurs spontaneously which gives rise to sporadic prion illnesses. In all cases of disease, as infectious PrP^{Sc} comes into contact with PrP^C, the normal PrP^C protein takes on the shape of PrP^{Sc}, a poorly understood process that nevertheless leads to an exponential increase of PrP^{Sc}. As the abnormal protein builds up, it forms into plaques known as amyloid which causes neuronal cell damage and eventually death. The loss of these cells creates vacuoles

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[1] Málaga-Trillo E, Sempou E. PrPs: Proteins with a purpose: Lessons from the zebrafish. *Prion*. 2009;3(3):129-33.
[2] Westergard L, Christensen HM, Harris DA. The cellular prion protein (PrP(C)): its physiological function and role in disease. *Biochim Biophys Acta*. 2007;1772(6):629-44.

within the brain, leading to the sponge-like neuronal tissue appearance characteristic of prion diseases.

Although every human has the precursor to the infectious prion protein, the likelihood for progression to disease is not uniformly distributed, as a polymorphism at codon 129 of the *PRNP* gene has been linked to prion disease susceptibility. The genotype of this codon is determined by the amino acid methionine/valine polymorphism, which creates 3 possible outcomes: homozygous methionine (MM), homozygous valine (VV), and heterozygous methionine/valine (MV). Homozygosity with either methionine or valine has been identified as a susceptibility risk[3,4] while heterozygosity has been associated with greater protection against most human prion diseases.[5]

[3] Kobayashi A, Hizume M, Teruya K, Mohri S, Kitamoto T. Heterozygous inhibition in prion infection: the stone fence model. *Prion*. 2009;3(1):27-30.
[4] Lloyd S, Mead S, Collinge J. Genetics of prion disease. Top Curr Chem. 2011;305:1–22.
[5] Bonda DJ, Manjila S, Mehndiratta P, et al. Human prion diseases: surgical lessons learned from iatrogenic prion transmission. *Neurosurg Focus*. 2016;41(1):E10.

Human Prion Diseases Today

Sporadic

sCJD

      Spontaneously occurring, or sporadic, cases are the most common forms of prion disease. Sporadic Creutzfeldt-Jakob Disease (sCJD) accounts for about 85% of all diagnosed cases of prion disease[6,7] with an estimated incidence of about 1-1.5 cases per million people per year globally.[8] There are 6 distinct clinical subtypes within the umbrella of sCJD, which combines 2 different types of PrP^{Sc} aggregation types along with the 3 possible codon 129 genotypes discussed above. The 2 PrP^{Sc} types are distinguished according to the banding patterns of PrP^{Sc} fragments that are resistant to proteinase K digestion.[9] The average age of onset for sCJD is 64 years with a mean disease duration of about 8 months[10] from when symptoms first begin to death, although extreme ranges from 1 to 72 months have been reported.[11] Typical symptoms of this disease include dementia, spasmodic muscle contraction (myoclonus), and cerebellar dysfunction.[5]

sFI

      By comparison, cases of sporadic fatal insomnia (sFI) are much rarer, typically having a younger age at onset and a longer median duration. Cases of sFI comprise about only 1-2% of all diagnosed cases of prion disease.[10,12] Compared to sCJD, which primarily affects older adults, sFI is more likely to affect those in middle age (median age of 46 years) and with an

[6] Elmallah MI, Borgmeyer U, Betzel C, Redecke L. Impact of methionine oxidation as an initial event on the pathway of human prion protein conversion. Prion. 2013;7:404–411.

[7] Prusiner SB. Molecular biology of prion diseases. Science. 1991;252:1515–22.

[8] Belay ED. Transmissible spongiform encephalopathies in humans. Annu Rev Microbiol. 1999;53:283–314.

[9] Cali I, Castellani R, Alshekhlee A, et al. Co-existence of scrapie prion protein types 1 and 2 in sporadic Creutzfeldt-Jakob disease: its effect on the phenotype and prion-type characteristics. *Brain*. 2009;132(Pt 10):2643-58.

[10] Puoti G, Bizzi A, Forloni G, Safar JG, Tagliavini F, Gambetti P. Sporadic human prion diseases: molecular insights and diagnosis. Lancet Neurol. 2012;11:618–628.

[11] Kong Q, Surewicz WK, Petersen RB, Zou WQ, Chen SG, Parchi P, et al. Inherited prion diseases. In: Prusiner SB, editor. Prion Biology and Disease. New York: Cold Spring Harbor Laboratory Press; 2004. pp. 673–775.

[12] Blase JL, Cracco L, Schonberger LB, et al. Sporadic fatal insomnia in an adolescent. *Pediatrics*. 2014;133(3):e766-70.

average duration of 24 months.[10] Insomnia and sleep disturbances are a characteristic feature of

this disease, with some cases reporting extreme myoclonic movements during sleep that would

result in occasional falls from bed.[13] A number of symptoms associated with cognitive decline

are also intrinsic to this disease, such as depression, forgetfulness and confusion, memory recall,

urinary incontinence, double vision, and mood changes. Unlike fatal familial insomnia, there is

no association between a mutation in the *PRNP* gene and this disease.[5]

VPSPr

Variably protease-sensitive prionopathy (VPSPr) is one of the more recently discovered

prion diseases and is also one of the rarest. Globally affecting only 2-3 people per 100 million

per year,[5] this disease is differentially affected by the codon 129 genotypes compared to sCJD,

according to one study.[10] Whereas sCJD incidence was greatest (70% of all sCJD cases) among

those with the homozygous methionine genotype, those with this genotype had the lowest (12%)

incidence among VPSPr cases. Conversely, the VV genotype was most common (62% of all

cases) in VPSPr cases while only 19% of those with sCJD had the homozygous valine genotype.

Both diseases had fairly low incidences of the protective heterozygous genotype, with sCJD and

VPSPr consisting of 11% and 26% MV genotypes, respectively. This finding implies that codon

129 acts as a different risk factor among both of these diseases. Median age of this disease more

closely resembles sCJD compared to sFI, with a typical age of illness at 70 years old, while

duration of illness resembles that of sFI at about 24 months. Mood changes, speech impairment,

and dementia characterize the early stages of this disease, while myoclonus and ataxia

characterize the later stages.[14,5]

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[13] Parchi P, Capellari S, Chin S, Schwarz HB, Schecter NP, Butts JD, et al. A subtype of sporadic prion disease mimicking fatal familial insomnia. Neurology. 1999;52:1757–1763.
[14] Zou WQ, Puoti G, Xiao X, et al. Variably protease-sensitive prionopathy: a new sporadic disease of the prion protein. *Ann Neurol*. 2010;68(2):162-72.

Genetic

fCJD

After sCJD, familial Creutzfeldt-Jakob Disease (fCJD) is one of the most common prion diseases, making up about 5-15% of all CJD cases.[15] As it is autosomal-dominantly inherited, the majority of fCJD cases have a family history of the disease with mutations in various codons in the *PRNP* gene.[8] Cases of fCJD tend to be younger than those with sCJD with an average age of onset at 58 years. However, the duration of the illnesses are similar (6 months). Typical symptoms include gait, speech, and visual abnormalities along with rapidly progressive neurological dysfunction and myoclonus.

FFI

Another autosomal-dominant disease, fatal familial insomnia (FFI) shares many characteristic traits of prion disease with sFI as the name suggests. The hallmark symptom of FFI, intractable insomnia, may begin with mild sleep difficulties that progressively worsen until the individual is not able to sleep at all. If sleep is achieved, it is only for a few hours at a time and filled with vivid dreams. Pinpoint pupils, profuse sweating, impotence, constipation, tremors, and eventually coma are also associated with this disease.[16,17] The key distinction between sFI and FFI is that the genetic disease is associated with *PRNP* mutations at D178N. D178N haplotype determines patient phenotype, with D178N-129M linked to FFI and D178N-129V linked to fCJD phenotype. Duration greatly varies according to codon 129 genotype. Those with the MV genotype report the longest duration of disease (approximately 23 months), lending credence to the assumption that this heterozygous genotype is least susceptible to

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[15] Gambetti P, Parchi P, Chen SG. Hereditary Creutzfeldt-Jakob disease and fatal familial insomnia. Clin Lab Med. 2003;23:43–64.
[16] Robson, D. The tragic fate of the people who stop sleeping. BBC. 2016. < http://www.bbc.com/future/story/20160118-the-tragic-fate-of-the-people-who-stop-sleeping>.
[17] Max, DT. The Family That Couldn't Sleep. Portobello Books Ltd. 2008.

disease. Among the two homozygous genotypes, those with dual methionine have an average

disease duration of about 11 months.[5,15] Given that the allele that that causes FFI is always

paired with methionine at codon 129, it is not possible to have a VV FFI patient. Put another

way, since FFI is linked only to methionine at D178N, a VV haplotype is not possible.

GSS

Exceedingly rare, Gerstmann-Straussler-Scheinker syndrome (GSS) is reported in 1-10

individuals per 100 million per year.[18] As it is associated with mutations in multiple codons,[19]

clinical manifestations of the disease may differ. It is, however, overwhelmingly associated with

a long duration of illness and non-rapid neurodegeneration unlike most other prion-related

diseases. Progression of the disease can extend anywhere from 3.5 to 9.5 years,[20] although

reports of shorter durations have been reported.[5] Onset typically occurs between 30 and 60 years

of age and symptoms include hyporeflexia, leg weakness, infrequent myoclonus, parkinsonian-

like signs, and gradual dementia.[20]

While associated with extensive amyloid plaque deposits, a rapidly progressive depletion

of neuronal cells has failed to be noted with GSS, leaving the brain tissue with only minimal

spongiform change. Like other genetic prion diseases, GSS is caused by mutations in codons on

the *PRNP* gene, although the frequency and distribution of these mutations varies across

nationalities.[21] Despite this unequal distribution of mutations, GSS incidence is uniform

worldwide.

[18] Liberski PP, Budka H. Gerstmann-Sträussler-Scheinker disease. I. Human diseases. Folia Neuropathol. 2004;42(Suppl B):120–140.
[19] Liberski PP, Surewicz WK. Molecular genetics of Gerstmann-Sträussler-Scheinker disease and Creutzfeldt-Jakob disease. Genetics. 2013;2:117.
[20] Imran M, Mahmood S. An overview of human prion diseases. Virol J. 2011;8:559.
[21] Jeong BH, Kim YS. Genetic studies in human prion diseases. J Korean Med Sci. 2014;29(5):623–32. doi: 10.3346/jkms.2014.29.5.623.

Acquired

iCJD

Cases of iatrogenic Creutzfeldt-Jakob Disease (iCJD) are comprised of individuals who were inadvertently infected with prion disease through instances of medical intervention. This type of acquired prion disease is most notably associated with contaminated cadaveric corneal transplants, dura mater grafts, or pituitary-derived human growth hormone along with those who had intracerebral contact with contaminated EEG needles or other neurosurgical instruments. iCJD is a relatively rare phenomenon in 2019; incidence curves of the disease peaked in the mid-1990s for those who were infected via contaminated growth hormone and in the late-1990s for those with contaminated dura mater, for example. As of 2012, these two routes of exposure were associated with the greatest number of iCJD cases, with dura mater linked to 228 cases and growth hormone linked to 226 cases. In comparison, all other sources of infection were associated with only a handful of cases combined, such as iCJD incidence due to neurological instruments (4) and EEG needles (2).[22] Depending on source of infection and route of exposure, clinical manifestations and average incubation times vary, although symptoms such as gait abnormalities and dementia are typical and incubation periods as short as 1 year and as long as 42 have been reported.[22,5]

As with other prion diseases, those who are homozygous at *PRNP* codon 129 represent a higher proportion of infected individuals with iCJD compared to those who are heterozygous. For example, 67% of iCJD cases from the United States, France, and the United Kingdom were homozygotic with an average incubation period of 13 years, while the remaining 33% of heterozygotes had a 17 year incubation period. Perhaps more dramatically, 96% of the 54 iCJD cases from Japan were homozygous but these were associated with an average 16 year

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[22] Brown P, Brandel JP, Sato T, et al. Iatrogenic Creutzfeldt-Jakob disease, final assessment. *Emerg Infect Dis*. 2012;18(6):901-7.

incubation period compared to the 13 year period in heterozygotes. As only 8% of the Japanese

population has the MV polymorphism, this surprisingly lower incubation period could be due to

the fact that this estimate arose from only a handful of individuals.[22]

vCJD

One of the most famous examples of acquired prion disease, peak incidence of variant

Creutzfeldt-Jakob Disease (vCJD) has fortunately appeared to have passed. Primarily stemming

from the consumption of beef contaminated with cow-specific prion disease known as bovine

spongiform encephalopathy, secondary human-to-human transmission has been reported via

blood transfusions and use of blood product. First cases of vCJD were reported in 1996 in the

United Kingdom and reached a peak annual incidence of 28 cases in 2000; as of 2017, there have

been 231 cases worldwide.[23,24] While only a handful of cases have been reported in the past

decade, millions of people were exposed to potentially contaminated beef during the epidemic.

Fortunately, there appears to be an interspecies barrier that is protective against vCJD via

the *PRNP* codon 129 polymorphism. Up until 3 years ago, all cases of this disease that

underwent genetic testing were homozygous with the methionine genotype. One case of the 129

MV genotype was identified in 2016.[24] It is possible that those with this genotype have a longer

incubation period for vCJD and so there will continue to be reported cases of the disease over the

upcoming years, however, much is unknown at this point.

Although it is not the sole prion disease to do so, vCJD is remarkable in that it typically

affects younger individuals, as the average age of onset is about 27 years, although cases as

young as 12 and as old as 74 years have been reported.[5,23] Mean duration is about 16 months and

symptoms consist of involuntary movement, cognitive impairment, and ataxia.[5,20,24] A

[23] Chen C, Dong XP. Epidemiological characteristics of human prion diseases. *Infect Dis Poverty*. 2016;5(1):47. Published 2016 Jun 2. doi:10.1186/s40249-016-0143-8.

[24] Knight R. Infectious and Sporadic Prion Diseases. *Progress in Molecular Biology and Translational Science*. 2017;293:318

characteristic feature of vCJD is the painful sensory symptoms cases undergo wherein sufferers experience limb pain, numbness, and cold feelings[24] along with psychiatric and behavioral changes.[5]

Diagnostic Tools

Conclusively diagnosing prion disease can be a difficult undertaking. The current gold standard of diagnosis via autopsy, wherein PrP^{Sc} is detected in the brain tissue, poses significant barriers to ascertaining disease status.[25] Unfortunately, family reluctance to provide consent to the procedure coupled with transmission concerns among hospitals and funerary services makes it difficult to definitively diagnose a prion disease.[26] Beyond these obstacles, autopsy is a less than ideal diagnostic tool as it requires the patient to have died in order to conduct histopathological examinations on a probable case's brain tissue.

Given the challenges and limitations that face postmortem diagnoses, a clear need for antemortem assays presents itself. Two such tests, 14-3-3 and tau, have been in use for decades. 14-3-3 is a protein often found in the cerebrospinal fluid (CSF) of patients with sporadic CJD, the most common prion disease. Since its discovery in 1986, 14-3-3 has been a helpful tool for clinicians in determining whether patients have sCJD, although the test performs poorly in capturing other types of prion disease.[27] Sporadic CJD sensitivity and specificity ranges for this assay are, respectively, 43-100% and 47-97%.[28,29]

Whereas 14-3-3 is more of a qualitative test, tau is quantitative. Tau has been shown to be a superior option to 14-3-3 as it is more sensitive and specific to the prion protein.[28] Other diseases such as Alzheimer's disease use tau as a marker for illness because it is a protein of neurofibrillary tangles, which are often the cause of neurodegeneration. Tau can be extracted from CSF which makes it a great option for antemortem diagnosis. While more sensitive and

[25] Maddox RA, Blase JL, Mercaldo ND, et al. Clinically Unsuspected Prion Disease Among Patients With Dementia Diagnoses in an Alzheimer's Disease Database. *Am J Alzheimers Dis Other Demen*. 2015;30(8):752-5.
[26] Belay ED, Holman RC, Schonberger LB. Creutzfeldt-Jakob disease surveillance and diagnosis. Clin Infect Dis. 2005;41(6):834–836.
[27] Green AJE. Use of 14-3-3 in the diagnosis of Creutzfeldt-Jakob disease. Biochem Society Trans. 2002;30(4):382-386.
[28] Hamlin C, Puoti G, Berri S, et al. A comparison of tau and 14-3-3 protein in the diagnosis of Creutzfeldt-Jakob disease. *Neurology*. 2012;79(6):547-52.
[29] Cuadrado-Corrales N, Jiménez-Huete A, Albo C, et al. Impact of the clinical context on the 14-3-3 test for the diagnosis of sporadic CJD. *BMC Neurol*. 2006;6:25. Published 2006 Jul 26. doi:10.1186/1471-2377-6-25

specific than 14-3-3 (82-87% and 67-71%, respectively),[28,30] tau is not a perfect test and often these diagnostic tools are used in tandem to ascertain disease likelihood, along with electroencephalogram (EEG) and brain magnetic resonance imaging (MRI). EEG was one of the first ancillary tests to diagnose sCJD. Periodic sharp-wave complexes indicate abnormalities among two-thirds of cases, although repeat testing is usually needed as these complexes only appear late in the disease course. MRI has been shown to be highly sensitive (92-96%) and specific (93-94%) for sCJD; unfortunately, the majority of MRIs for positive cases are either misread by radiologists or the abnormalities are failed to be reported.[31]

Real-time quaking-induced conversion (RT-QuIC) debuted in 2011 as an *in vitro* tool in which prion proteins, taken from CSF, are amplified. More specifically, RT-QuIC takes advantage of the self-replicating, or seeding, nature of the misfolded PrP^{Sc}. A recombinant form of PrP^{C} that is protease sensitive ($rPrP^{Sen}$, taken from hamster brains) is added as a substrate along with very small amounts of PrP^{Sc} seed from the CSF of a potential case.[30] N-terminal truncated hamster $rPrP^{Sen}$ (residues 90-231) strengthens RT-QuIC results as it is more efficient at speeding up prion reactivity compared to other models while also generating more robust results compared to other human and animal substrate models.[32] However, RT-QuIC prion strain discrimination has been linked to the use of different types of substrates, indicating that some may be better than others at promoting certain PrP^{Sc} amplification. However, it is unknown whether these differences reflect abnormal PrP concentrations, strain-specific PrP^{Sc} properties, or both.[33]

[30] Foutz A, Appleby BS, Hamlin C, et al. Diagnostic and prognostic value of human prion detection in cerebrospinal fluid. *Ann Neurol*. 2017;81(1):79-92.
[31] Geschwind MD. Prion Diseases. Continuum (Minneap Minn). 2015;21(6 Neuroinfectious Disease):1612–1638.
[32] Orrú CD, Hughson AG, Groveman BR, et al. Factors That Improve RT-QuIC Detection of Prion Seeding Activity. *Viruses*. 2016;8(5):140. Published 2016 May 23. doi:10.3390/v8050140
[33] Franceschini A, Baiardi S, Hughson AG, et al. High diagnostic value of second generation CSF RT-QuIC across the wide spectrum of CJD prions. *Sci Rep*. 2017;7(1):10655. Published 2017 Sep 6. doi:10.1038/s41598-017-10922-w

In the second generation of RT-QuIC, a fluorescent dye known as thioflavin T (ThT) was added to the rPrP^{Sen} and PrP^{C} mixture in order to detect prion seeding of rPrP^{Sen} polymerization, which resulted in a faster and more sensitive test.[30,34] Any PrP^{Sc} that is present in the CSF will bind to the rPrP^{Sen} when the mixture undergoes repeated shake/rest cycles. The PrP^{Sc} will induce the protease sensitive protein to convert from its monomeric and α-helix rich form to a protease resistant, multimeric, and β-sheet rich form, a process that leads to the creation of PrP^{Sc} fibrils.[35] This process of fibril formation is known as the lag phase and can take up to 30 hours.

After the lag phase, the fibrils aggregate and bind to the ThT which begins to fluoresce and is monitored in real time.[36] Fluorescence allows these misfolded proteins to be detected.[37,38] Total reaction time can take up to 60 hours, with alternating 60 second shaking and resting periods (continuous shaking promotes false positive RT-QuIC results).[30,32] ThT measurements are taken every 45 minutes.[30]

Second generation RT-QuIC has the highest predictive values of any test, with sensitivity and specificity for second generation RT-QuIC approaching 100%.[30,33,36] While autopsy is still considered to be the gold standard even with the introduction of this novel assay, RT-QuIC has made it possible for clinicians to more confidently diagnose their patients with prion disease. Evaluation of this assay's performance is key in diagnosing prion disease antemortem as knowledge of a patient's disease status may help in seeking care and potential experimental treatments.

[34] Wilham JM, Orrú CD, Bessen RA, et al. Rapid end-point quantitation of prion seeding activity with sensitivity comparable to bioassays. *PLoS Pathog*. 2010;6(12):e1001217. Published 2010 Dec 2. doi:10.1371/journal.ppat.1001217

[35] Orrú CD, Groveman BR, Hughson AG, Zanusso G, Coulthart MB, Caughey B. Rapid and sensitive RT-QuIC detection of human Creutzfeldt-Jakob disease using cerebrospinal fluid. *MBio*. 2015;6(1):e02451-14. Published 2015 Jan 20. doi:10.1128/mBio.02451-14

[36] Green AJE. RT-QuIC: a new test for sporadic CJD. Practical Neurology 2019;19:49-55.

[37] Atarashi R, Satoh K, Sano K, Fuse T, Yamaguchi N, Ishibashi D, et al. Ultrasensitive human prion detection in cerebrospinal fluid by real-time quaking-induced conversion. Nature medicine. 2011;17(2):175–8. Epub 2011/02/01. 10.1038/nm.2294 .

[38] Takatsuki H, Satoh K, Sano K, et al. Rapid and Quantitative Assay of Amyloid-Seeding Activity in Human Brains Affected with Prion Diseases. *PLoS One*. 2015;10(6):e0126930. Published 2015 Jun 12. doi:10.1371/journal.pone.0126930

Additionally, since prion diseases affect only about 1 in every million individuals per year, most clinicians do not have experience in diagnosing these kinds of diseases and must rely on a test that can accurately capture true cases of the disease. As prions are invariably fatal, such a diagnosis is usually a last result for clinicians; every other possibility must be ruled out. Therefore, the test must also be highly specific to exclude those who are truly negative for the disease. In summary, a diagnostic test must be as close to perfect as possible in order for clinicians to have faith in the results.

CURRENT STUDY

Given the many phenotypes and clinical manifestations of prion disease, coupled with the fact that the diseases are so rarely encountered, clinicians and researchers have been challenged to ascertain confident diagnoses. Prion diseases are universally distributed and uniformly affect about 1-2 new cases per million individuals; until recently diagnostic technologies included 14-3-3 and tau, along with specific electroencephalogram (EEG) and brain magnetic resonance imaging (MRI) changes. As of September 2018, the Centers for Disease Control and Prevention (CDC) added RT-QuIC as a reliable diagnostic tool for sporadic CJD.[39]

About 500 incident cases of prion disease are identified each year in the United States.[40] The National Prion Disease Pathology Surveillance Center (NPDPSC) in Cleveland, Ohio was created in order to assist the CDC surveil this group of diseases using autopsy findings and death certificate data. This observational study will describe the NPDPSC's experience with 2nd generation RT-QuIC during its first three years of use as a diagnostic test. From May 2015 to April 2018, over 10,000 CSF samples from cases of suspected prion disease were sent to the NPDPSC for testing. The demographics of this group along with the more than 1,000 individuals with positive RT-QuIC results will be described. Evaluation of the accuracy of this test will be compared to a subset of cases that underwent antemortem gold standard autopsy.

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[39] Centers for Disease Control and Prevention. Diagnostic Criteria. 2018. <https://www.cdc.gov/prions/cjd/diagnostic-criteria.html>.
[40] Holman RC, Belay ED, Christensen KY, et al. Human prion diseases in the United States. *PLoS One*. 2010;5(1):e8521. Published 2010 Jan 1. doi:10.1371/journal.pone.0008521

METHODS

Subject and specimen selection

During the 36-month period between May 1, 2015 and April 31, 2018, the NPDPSC received 11,016 CSF samples from cases of suspected prion disease around the United States. Of these, only subjects' first CSF specimen that produced a positive or negative RT-QuIC result was included in the study (n=10,498). Accession date is determined by the day the CSF specimen was received at the NPDPSC, usually about 1 week after the sample was collected. Those within the autopsy cohort were referred for autopsy through the NPDPSC's Autopsy Coordination Program and were included in this cohort if the NPDPSC received relevant tissue within the study time frame.

Antemortem CSF Testing

Total tau, 14-3-3, and 2nd generation RT-QuIC testing were performed on CSF specimens. Total tau was measured by a quantitative ELISA (Life Technologies, Carlsbad, CA); 14-3-3 was evaluated qualitatively by Western blot using an anti-14-3-3 beta antibody (Abcam, Cambridge, MA); 2nd generation RT-QuIC was performed as previously described.[30] N-terminally truncated Syrian hamster recombinant prion protein [SharPrP(90-231)] was used as the rPrP^{Sen} substrate, as animal models have shown this type of hamster to be more efficient at rapidly propagating certain prion strains compared to other models.[32,41] Type of substrate used influences the assay's ability to discern certain prion diseases but the 90-231 substrate is conducive to detect the most common forms of prion disease, such as sCJD in humans and chronic wasting disease and scrapie in animals.[32]

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[41] Brandner S, Jaunmuktane Z. Prion disease: experimental models and reality. *Acta Neuropathol.* 2017;133(2):197–222. doi:10.1007/s00401-017-1670-5

Based on previous empiric findings, tau concentrations less than 500 pg/ml are generally not suggestive of prion disease.[28] Concentrations 500-1149 pg/ml have been considered elevated but lower than what is typically observed in prion disease. Concentrations of 1150-2499 pg/ml have been considered elevated and concerning for prion disease, and concentration of 2500 pg/ml or greater have been considered highly suggestive of prion disease.

These three tests were intended to be performed on all subjects' CSF specimens. However, bloody specimens can yield falsely positive 14-3-3 results and falsely negative RT-QuIC results. Therefore, subjects whose specimens were not tested by RT-QuIC due to excessive bloodiness were excluded from the study. In the analyses, tau and 14-3-3 results were obtained from the same specimen as the RT-QuIC result.

Autopsy Evaluation

Subjects whose fixed or frozen autopsy tissue samples were received were included in the autopsy subgroup. Samples underwent neuropathologic examination and prion protein detection by Western blot test. Frozen tissue samples were able to be genetically characterized (e.g. sCJD MM1) and qualitatively diagnosed, while fixed tissue samples were only able to be qualitatively diagnosed (e.g. prion disease NOS).[42] Genetic testing of the *PRNP* gene and codon 129 polymorphism was conducted by University Hospitals Cleveland Medical Center Department of Genetics.

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[42] Parchi P, Giese A, Capellari S, et al. Classification of sporadic Creutzfeldt-Jakob disease based on molecular and phenotypic analysis of 300 subjects. Annals of Neurology 1999;46(2):224–33.

Statistical Analysis

Frequency analysis was used to evaluate the demographics among the study populations across RT-QuIC results. P-values were derived from chi-square calculations for cell counts greater than five; otherwise, Fischer's exact test was used.

Sex, CSF sample quality (clear vs. slightly bloody), and age (continuous) variables were all included in multivariate binary logistic regression models in order to examine factors that affect the sensitivity and false negativity RT-QuIC results. Sensitivity analysis was only conducted on those who were diagnosed with sCJD, with polymorphism (MM, MV, VV) included as a factor. In the false negativity analysis, 14-3-3 results (positive vs. negative) and tau (<500, 500-1149, 1150-2499, and >2499) were also included. Two-sided Type I error level was 0.05.

SAS version 9.4 and IBM SPSS Statistics version 25 were used in conducting the analyses. This study was approved by the University Hospitals Cleveland Medical Center Institutional Review Board.

RESULTS

A total of 10,778 unique individuals contributed 11,016 CSF samples to the National Prion Disease Pathology Surveillance Center between May 1, 2015 and April 31, 2018. Subjects' first samples that produced a positive or negative result were included; 238 (2.2%) samples were excluded as they did not meet this criterion. Of those excluded, 196 samples (1.8%) were excluded due to poor CSF sample quality and 84 (0.8%) were not included due to inadequate CSF volume, test order cancellation, indeterminate results, or unavailable results. A total of 10,498 specimens were therefore included in the study. Of these, 567 (5.4%) contributed autopsy tissue samples that were analyzed by the NPDPSC; 497 (87.7%) were positive for prion disease and 70 (12.3%) were negative. Of the 497 positive cases, 439 (88.3%) were diagnosed as sCJD with subtype, 38 (7.6%) with genetic prion disease, 124 (2.4%) with prion disease NOS, 5 (1.0%) with sFI, 3 (0.6%) with VPSPr, and none with acquired prion disease.

Among the 10,498 RT-QuIC results, 9,395 (89.5%) were negative and 1,103 (10.5%) were positive. The demographics and laboratory results of these two groups are listed in Table 1. The mean age of subjects was older in the RT-QuIC positive groups. Sex was not found to be indicative of RT-QuIC positivity at the $p=0.05$ level of significance. The majority of samples had missing race and ethnicity data but among subjects that had this information available, non-Hispanic white individuals were more likely to be RT-QuIC positive compared to other ethnic groups ($p<0.001$). Total tau concentration was higher in RT-QuIC positive specimens than in RT-QuIC negative specimens and 14-3-3 was more likely to be positive in RT-QuIC positive specimens than in RT-QuIC negative specimens ($p<0.001$). Both 14-3-3 and RT-QuIC positivity independently correlated with increased tau concentrations but RT-QuIC positivity correlated with a greater magnitude of tau positivity than 14-3-3 (Table 1).

Table 1. Description of the Entire Sample, by RT-QuIC Results*			
Characteristic	Positive (N = 1,103)†	Negative (N = 9,395)†	p value‡
Age (years)	67.4 ± 9.4	64.7 ± 13.5	<0.001
Male	559 (50.9)	4,880 (52.8)	0.234
Ethnicity			<0.001
Hispanic/Latino	34 (3.1)	138 (1.5)	
Non-Hispanic/Latino	25 (2.3)	51 (0.5)	
Unknown	1,044 (94.7)	9,206 (98.0)	
Race			<0.001
White	597 (54.1)	2,840 (30.2)	
Black	31 (2.8)	375 (4.0)	
Asian	21 (1.9)	83 (0.9)	
Native American	6 (0.5)	14 (0.2)	
Other	52 (4.7)	189 (2.0)	
Unknown	396 (35.9)	5,894 (62.7)	
Total tau (pg/mL)			<0.001
<500	32 (2.9)	5,791 (61.6)	
500-1,150	47 (4.3)	2,290 (24.4)	
1,151-2,499	134 (12.1)	667 (7.1)	
>2,499	890 (80.7)	647 (6.9)	
14-3-3 positive	920 (83.4)	2,334 (24.8)	<0.001

* Table values are mean ± SD for continuous variables and n (column %) for categorical variables.

† Numbers may not sum to total due to missing data, and percentages may not sum to 100% due to rounding.

‡ P-value is for analysis of variance F-test (continuous variables) or $x2$ test (categorical variables).

As prion disease affects 1-2 out of every million individuals across all populations, incidence rate should be stable across all regions. This study therefore also set out to measure the RT-QuIC testing rates within the United States in order to determine which states were failing to surveil or detect suspected cases. States' population sizes were adjusted using the United States Census Bureau's estimated population for 2017. Testing rates for the 10,498 sample specimens submitted to the NPDPSC during the study time period per 100,000

individuals are shown below in Figure 1. Upon separating the data into quintiles, a total of 19

states were found to be below the average threshold of specimen submission.

Figure 1. RT-QuIC Testing Rate per 100,000 Individuals
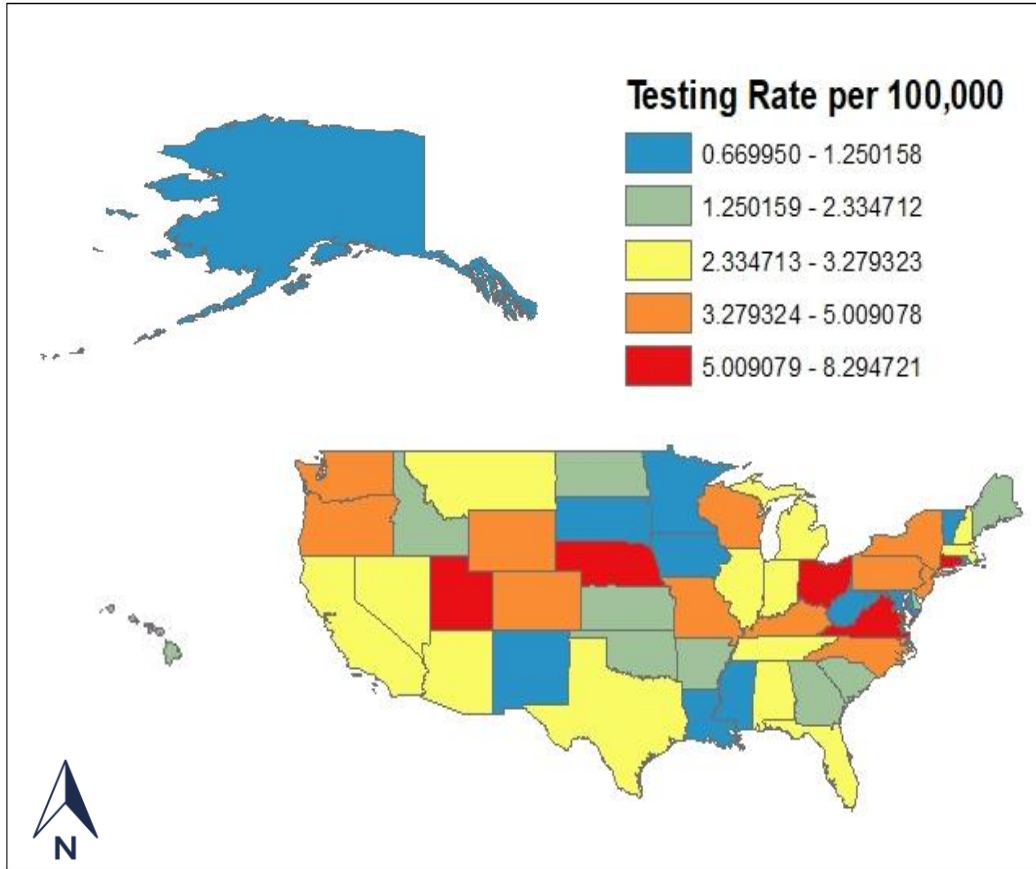
Of the 567 cases that had autopsy performed, 117 (20.6%) had negative RT-QuIC results

prior to their death and of these, 48 (41%) were positive for prion disease by autopsy. Among

the 450 cases with positive RT-QuIC results, 449 (99.8%) were positive for prion disease by

autopsy. One specimen had a false positive RT-QuIC result; final diagnosis of this case was

multifactorial dementia (Alzheimer's disease and vascular).

The overall sensitivity and specificity of RT-QuIC for all autopsy cases diagnosed as a prion disease was 90.3% and 99.8%, respectively. RT-QuIC sensitivity was greatest in the most common forms of sporadic prion disease (96.3% for sCJD MM1 and VV2) and genetic prion disease (97%, fCJD). Comparatively, RT-QuIC did not detect any cases of either sporadic fatal insomnia or fatal familial insomnia (n=9). False negative RT-QuIC cases tended to be younger, had longer disease duration from onset to death, had longer onset to specimen accession duration, and longer specimen accession to death duration. The demographics of the 566 subjects within the autopsy subgroup (excluding the 1 RT-QuIC false positive case) are described in Table 2.

Characteristic	True RT-QuIC Positives (n=449)‡	False RT-QuIC Negatives (n=48)‡	True RT-QuIC Negatives (n=69)‡	p value§
Age (years)	66.7 ± 8.3	58.2 ± 9.8	66.4 ± 10.5	<0.001
Male	236 (53)	34 (71)	38 (55)	0.083
Ethnicity				0.599
Hispanic/Latino	13 (2.9)	1 (2.1)	0 (0.0)	
Non-Hispanic/Latino	18 (4.0)	1 (2.1)	2 (2.9)	
Unknown	418 (93.1)	46 (95.8)	67 (97.1)	
Race				0.176
White	358 (79.7)	40 (83.3)	50 (72.5)	
Black	7 (1.6)	1 (2.1)	5 (7.2)	
Asian	5 (1.1)	1 (2.1)	0 (0.0)	
Native American	2 (0.4)	0 (0.0)	0 (0.0)	
Other	18 (4.0)	1 (2.1)	1 (1.4)	
Unknown	59 (13.1)	5 (10.4)	13 (18.8)	
Total tau (pg/mL)				<0.001
<500	4 (0.9)	9 (18.8)	27 (39.1)	
500-1,150	17 (3.8)	11 (22.9)	18 (26.1)	
1,151-2,499	58 (12.9)	9 (18.8)	10 (14.5)	
>2,499	370 (82.4)	19 (39.6)	14 (20.3)	
14-3-3 positive	373 (83.1)	18 (37.5)	42 (60.9)	<0.001
Mean time from onset to accession; median (days)	143.6 ± 203.3; 85	272.5 ± 746.1; 139	165.3 ± 353.2; 104	0.028
Mean time from accession to death; median (days)	63.8 ± 101; 27	160.4 ± 208; 96	64.8 ± 122; 23	<0.001
Mean time from onset to death; median (days)	207.3 ± 248.2; 119	437.5 ± 870.1; 261	222.7 ± 339.8; 140	<0.001
Case Diagnosis				<0.001
Positive⚹	449 (100)	48 (100)	0 (0)	
Sporadic				
sCJD	408 (90.9)	31 (64.6)		
sFI	0 (0.0)	5 (10.4)		
VPSPr	2 (0.5)	1 (2.1)		
Genetic				
gCJD	30 (6.7)	1 (2.1)		
FFI	0 (0.0)	4 (8.3)		
GSS	1 (0.2)	2 (4.2)		
Negative	0 (0.0)	0 (0.0)	69 (100.0)	

Table 2. Description of Sub-Sample that Underwent Autopsy*,†

* Table values are mean ± SD for continuous variables and n (column %) for categorical variables.

Demographics, laboratory results, and *PRNP* codon 129 polymorphism characterization of the 439 sCJD cases that underwent autopsy are described in Table 3. Similar to above, false negatives tended to be younger, have longer disease duration, and have a longer duration between CSF specimen collection and death compared to specimens with true positive RT-QuIC results ($p < 0.05$). In addition, false negative RT-QuIC cases were more likely to have lower CSF total tau levels and were more likely to be positive for 14-3-3 ($p < 0.001$). Overall, *PRNP* codon 129 polymorphisms did not correlate with RT-QuIC testing accuracy, although certain molecular subtypes (MM2 and VV1) had a higher likelihood of producing a false negative RT-QuIC result.

Table 3. Description of sCJD Sample (n=439)*			
Characteristic	**RT-QuIC positive (n=408)†**	**RT-QuIC negative (n=31)†**	**p-value‡**
Age (years)	67.4 ± 8.4	61.2 ± 9.3	<0.001
Male	214 (52.5%)	21 (68%)	0.1
Ethnicity			0.922
Hispanic/Latino	10 (2.5)	1 (3.2)	
Non-Hispanic/Latino	18 (4.4)	1 (3.2)	
Unknown	380 (93.1)	29 (93.5)	
Race			0.731
White	325 (79.7)	24 (77.4)	
Black	7 (1.7)	0 (0.0)	
Asian	3 (0.7)	1 (3.2)	
Native American	2 (0.5)	0 (0.0)	
Other	15 (3.7)	1 (3.2)	
Unknown	56 (13.7)	5 (16.1)	
Total tau (pg/mL)			<0.001
<500	4 (1.0)	2 (6.5)	
500-1,150	17 (4.2)	7 (22.6)	
1,151-2,499	52 (12.7)	7 (22.6)	
>2,499	335 (82.1)	15 (48.4)	
14-3-3 positive	337 (82.6)	14 (45.2)	<0.001
Mean time from onset to accession; median (days)	143 ± 191; 88	156 ± 125; 126	0.732
Mean time from accession to death; median (days)	65 ± 103; 27.5	149 ± 199; 91	0.026
Mean time from onset to death; median (days)	208 ± 239; 122	308 ± 248; 248	0.034
sCJD Subtype			<0.001
MM1	182 (44.6)	7 (22.6)	
MM1-2	39 (9.6)	3 (9.7)	
MM2	18 (4.4)	5 (16.1)	
MV1	13 (3.2)	3 (9.7)	
MV1-2	56 (13.7)	5 (16.1)	
MV2	37 (9.1)	3 (9.7)	
VV1	0 (0.0)	3 (9.7)	
VV1-2	9 (2.2)	0 (0.0)	
VV2	54 (13.2)	2 (6.5)	
PRNP Codon 129			0.472
MM	239 (58.6)	15 (48.4)	
VV	63 (15.4)	5 (16.1)	
MV	106 (26.0)	11 (35.5)	

* Table values are mean ± SD for continuous variables and n (column %) for categorical variables.

† Numbers may not sum to total due to missing data, and percentages may not sum to 100% due to rounding.

‡ P-value is for analysis of variance F-test (continuous variables) or x2 test (categorical variables).

Multivariate Analyses of Factors Determining RT-QuIC Positivity

Logistic regression was applied to RT-QuIC binary outcome (positive vs. negative) among the autopsy confirmed prion positive subgroup (n=497). Sex, age, and sample quality variables were all found to be statistically significant for RT-QuIC results at p=0.05. Females were more likely to have a positive RT-QuIC result compared to males. Older subjects (OR[95%CI]: 1.115 [1.075,1.157]) were more likely to be positive than younger subjects. Sample quality was borderline significant, with clear samples about 65% more likely to be positive by RT-QuIC than not clear samples. Odds ratio and p-values are reported in Table 4 below.

Table 4. RT-QuIC Positivity Among Autopsy-Confirmed Positives (n=497)			
Parameter	**Adjusted Value**	**(95% CI)**	**P value**
Sex - Female	2.522	(1.260, 5.043)	0.009
Specimen quality - Not clear	0.349	(0.113, 1.077)	0.067
Age - Per unit increase	1.115	(1.075, 1.157)	<0.001

Factors That Predict Prion Disease in RT-QuIC Negative Samples

A similar logistic regression model was applied to a subsample of RT-QuIC negative results among autopsy-confirmed positive prion cases (n=94). As with the model above, sex, age, and specimen quality variables were included along with 14-3-3 binary results and tau level variables. Among this false negative sample, 14-3-3 negative results were more likely to be positive for prion disease (OR[95%CI]: 76.02 [4.486, 1286.91]). Using 500-1,1150 pg/ml as the reference category, those with 1,151-2,499 (OR[95%CI]: 21.456 [1.249, 368.706]) and greater than 2,499 (OR[95%CI]: 43.598[2.179, 872.184]) pg/ml total tau were statistically significantly more likely to be positive for prion disease. Clear samples were more likely to be negative for prion disease than not clear samples (OR[95%CI]: 47.942 [2.754, 835.475]). Age was also found to be significant, with every yearly unit increase in age associated with an 11% reduced likelihood of prion positivity. Model variables, odds ratios, and p-values are shown in Table 5.

Table 5. Prion Positivity Among RT-QuIC Negatives (n=94)			
	Autopsy Positive Result		
Parameter	**Adjusted Value**	**95% CI**	**P value**
Sex – Female	0.557	(0.159, 1.958)	0.362
Specimen quality - Not Clear	47.942	(2.754, 835.475)	0.008
Tau - <500	0.201	(0.039, 1.034)	0.055
Tau - 1151-2499	21.456	(1.249, 368.706)	0.035
Tau - >2499	43.598	(2.179, 872.184)	0.014
14-3-3 - Negative	76.02	(4.486, 1286.91)	0.003
Age - Per unit increase	0.889	(0.827, 0.955)	0.001

Total Positive and Probable Cases

Total annual number of positive and probable cases are shown in Table 6. In 2016 through 2018, a total of 467, 492, and 498 cases, respectively, were identified through RT-QuIC. Results from 2018 extend beyond the close of the study period described here.

Table 6. Number of Probable and Definite Prion Disease Cases Submitted to the Prion Center			
Year*	Prion Disease by Neuropathology	CSF RT-QuIC (+) Cases	Probable† & Definite Prion Disease
2016	278	356	467
2017	266	395	492
2018‡	209	420	498

* Listed based on year of the patient's first CSF submission

† RT-QuIC positive without neuropathologic examination

‡ This table includes 2018 surveillance data that extends beyond the close of
  the study period described in this article.

Updated table can be found at https://case.edu/medicine/pathology/divisions/national-prion-disease-pathology-surveillance-center/resources-for-professionals/tables-of-cases-examined-0

Since the NPDPSC began using 2nd generation RT-QuIC in 2015, case ascertainment has increased by about 93% compared to previous years. Not including death certificate data, incidence of prion disease is 1.55 per million in 2018. Incidence rates are shown in Figure 2 below.

Figure 2.

Incidence of Prion Disease Per Million: Autopsy Confirmed vs. Probable and Definite Prion Disease Cases
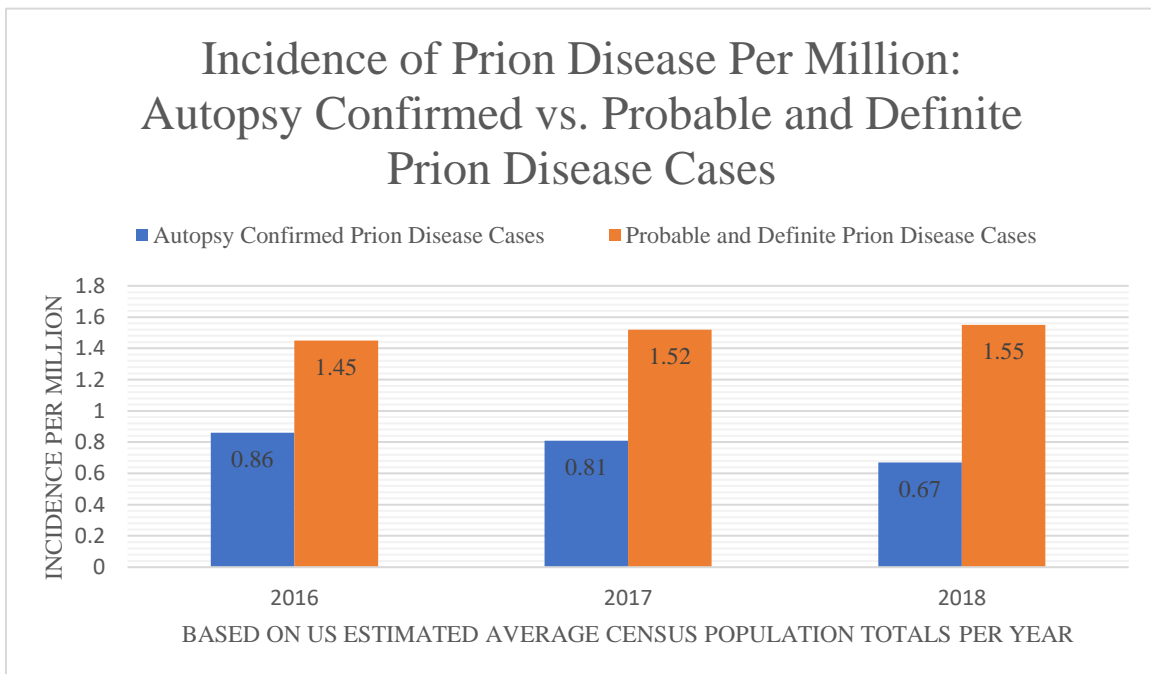
DISCUSSION

RT-QuIC has emerged as a highly sensitive and specific tool for diagnosing the most common forms of prion disease. While brain tissue biopsy remains the gold standard for diagnosis, RT-QuIC has been shown to be a powerful antemortem test for suspected cases of disease. This study describes the demographics, RT-QuIC results, and other laboratory results of 10,498 unique cases of suspected prion disease submitted to the National Prion Disease Pathology Surveillance Center, one of the largest such sample cohorts to be analyzed. RT-QuIC sensitivity and specificity was validated and found to be consistent with rates reported by other, smaller studies.[30,33,34] Both estimates approached 100% perfection overall, with 90.3% sensitivity and 99.8% specificity. Sensitivity estimates were highest for the most common types of prion disease and prion disease subtypes, such as sCJD and MM1, respectively. Strain variability most likely influenced false negative RT-QuIC results, possibly because of the assay's reduced matching to the recombinant seed. These false negative results were more likely in genetic prion diseases (ie., FFI and GSS) and atypical sporadic prion disease subtypes (i.e., sFI, VPSPr, VV1, and MM2). Longer disease duration and younger age during disease were other factors that contributed to lower RT-QuIC sensitivity, although this may be in large part due to the rarer prion diseases these factors are usually associated with. Use of this novel assay has increased case ascertainment by 93% and prion disease incidence is 1.55 cases per million according to the most recent annual data. This estimate is consistent with estimates widely reported in the literature.

As Figure 1 shows, increased surveillance is needed in rural settings that may currently be overlooking disease incidence, even after adjusting for population size. The majority of the lowest performing states in terms of testing tend to be located in the mid-West and Great Plains

regions of the United States. These areas are typically underserved by doctors and health care specialists[43] which likely influenced the poorer testing rates. Given the sparse populations in many of these settings, clinicians may be ill-equipped to recognize a prion disease when a case presents itself.

CDC guidelines now include positive RT-QuIC results as being a likely indicator of prion disease, and the continued use of this test will only serve to improve human prion disease surveillance efforts in the future. Only a small portion of suspected prion diseases go on to autopsy (just 5% of the study's cohort underwent the procedure), and current human prion epidemiology largely relies on death certificates that are subject to inaccuracies. Therefore, the adoption of a highly sensitive and specific assay of disease diagnosis will positively impact public health surveillance and disease ascertainment measures.

As has been mentioned, however, autopsy remains the gold standard of prion diagnosis, especially since RT-QuIC often fails to detect disease among less common disease types and subtypes. Neuropathologic examination is a much more powerful tool in determining disease etiology (i.e., sporadic, iatrogenic, or variant) and so is more capable at detecting novel prion diseases. As the spread of chronic wasting disease among cervids in the United States continues to grow,[44] and as these deer, mule deer, and elk continue to have greater contact with human populations, autopsy will remain the most useful tool in detecting these potentially new prion diseases.

The biggest limitation of this study was that only a small portion of the total number of cases went on to autopsy. As final diagnoses are contingent upon this procedure, this study may

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[43] University of Medicine and Health Sciences. Medically Underserved Areas in the US. 2013. < https://www.umhs-sk.org/blog/medically-underserved-areas-regions-where-u-s-needs-doctors/>.

[44] Centers for Disease Control and Prevention. Occurrence. 2019. <https://www.cdc.gov/prions/cwd/occurrence.html>.

have missed false positive RT-QuIC cases that did not proceed to autopsy, or false negative cases wherein prion disease was deemed clinically unlikely after a negative RT-QuIC result. Implicit bias may have also affected the autopsy cohort study results, as cases that went on to autopsy through the NPDPSC Autopsy Program were likely to be prion cases. Another limitation is that certain cases had missing home addresses and the address used was that of CSF accession facility, so testing rate reporting may not be entirely accurate. In addition, the study sample contained only a limited number of unusual or rare prion diseases or disease subtypes. Future studies are recommended to examine in greater detail the effect of different recombinant protein substrates may have on RT-QuIC results among these rarer prion disease strains. In addition, more research is needed in order to determine whether RT-QuIC sensitivity is affected by age, disease duration, and time from onset to specimen collection, or whether it is instead influenced by atypical subtypes that are more likely to have a longer disease course.

CONCLUSION

Previous studies have reported RT-QuIC to be a highly sensitive and specific diagnostic tool for prion diseases, results which were validated and replicated in this large observational study. While RT-QuIC results vary by disease type, specimen quality, and demographic characteristics among individuals with suspected prion disease, this paper has shown that this novel assay is an invaluable objective tool in diagnosing prion disease antemortem. As chronic wasting disease continues to spread among cervids in the United States, prion surveillance will only become more vital. RT-QuIC is a useful tool in this direction although future research needs to be done concerning its use with rarer prion disease types. With clinicians now able to more confidently diagnose prion disease prior to the death of an afflicted individual, there is reason to hope these individuals may someday enroll in potential prion disease treatment studies.

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