Diagnosis of prion diseases by RT-QuIC results in improved surveillance

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Abstract

Objective
To present the National Prion Disease Pathology Surveillance Center’s (NPDPSC’s) experience using CSF real-time quaking-induced conversion (RT-QuIC) as a diagnostic test, to examine factors associated with false-negative RT-QuIC results, and to investigate the impact of RT-QuICs on prion disease surveillance.

Methods
Between May 2015 and April 2018, the NPDPSC received 10,498 CSF specimens that were included in the study. Sensitivity and specificity analyses were performed on 567 autopsy-verified cases. Prion disease type, demographic characteristics, specimen color, and time variables were examined for association with RT-QuIC results. The effect of including positive RT-QuIC cases in prion disease surveillance was examined.

Results
The diagnostic sensitivity and specificity of RT-QuIC across all prion diseases were 90.3% and 98.5%, respectively. Diagnostic sensitivity was lower for fatal familial insomnia, Gerstmann-Sträussler-Scheinker disease, sporadic fatal insomnia, variably protease sensitive prionopathy, and the VV1 and MM2 subtypes of sporadic Creutzfeldt-Jakob disease. Individuals with prion disease and negative RT-QuIC results were younger and had lower tau levels and nonelevated 14-3-3 levels compared to RT-QuIC-positive cases. Sensitivity was high throughout the disease course. Some cases that initially tested RT-QuIC negative had a subsequent specimen test positive. Including positive RT-QuIC cases in surveillance statistics increased laboratory-based case ascertainment of prion disease by 90% over autopsy alone.

Conclusions
RT-QuIC has high sensitivity and specificity for diagnosing prion diseases. Sensitivity limitations are associated with prion disease type, age, and related CSF diagnostic results. RT-QuIC greatly improves laboratory-based prion disease ascertainment for surveillance purposes.

Classification of evidence
This study provides Class III evidence that second-generation RT-QuIC identifies prion disease with a sensitivity of 90.3% and specificity of 98.5% among patients being screened for these diseases due to concerning symptoms.
Glossary

CI = confidence interval; NPDPSC = National Prion Disease Pathology Surveillance Center; OR = odds ratio; RT-QuIC = real-time quaking-induced conversion; sCJD = sporadic Creutzfeldt-Jakob disease.

Prion disease is an untreatable, fatal, and rapidly progressive neurodegenerative disease. The worldwide annual incidence is 1 to 2 new cases per million individuals. Making a confident diagnosis of prion disease can be challenging because it is rarely encountered, has ecletic phenotypes, and has many clinical mimickers. Until recently, objective diagnostic studies were limited to EEG and the evaluation of nonspecific neuronal protein concentrations in the CSF (e.g., 14-3-3 and tau). Better diagnostic technologies are now available such as brain MRI and real-time quaking-induced conversion (RT-QuIC). RT-QuIC exploits the autocatalytic template-directed protein misfolding that occurs in prion diseases, thereby amplifying minute amounts of prions to enable their detection. Slight changes to the original assay increased its sensitivity and have been previously described (e.g., second-generation RT-QuIC).

The National Prion Disease Pathology Surveillance Center (NPDPSC) is funded by the Centers for Disease Control and Prevention to assist in the surveillance of the US population for prion diseases. Historically, surveillance has relied on autopsy findings reported by the NPDPSC and death certificate data. Similar to the UK’s National CJD Research & Surveillance Unit, the Centers for Disease Control and Prevention added RT-QuIC to the diagnostic criteria for sporadic Creutzfeldt-Jakob disease (sCJD) in September 2018. Beginning in 2019, the NPDPSC began reporting RT-QuIC results on all CSF specimens submitted to the NPDPSC for diagnostic testing. This study reinforces why changes in diagnostic criteria and reporting metrics are appropriate and innovative in the diagnosis and surveillance of prion disease.

In this observational study, we describe the NPDPSC’s experience with second-generation RT-QuIC during the first 3 years of its use as a diagnostic test. We describe the demographics of the >10,000 individuals whose CSF specimens were tested during this period and the demographics of the >10,000 individuals with positive RT-QuIC results. We also evaluate the accuracy of RT-QuIC in a subset of individuals who subsequently came to the NPDPSC for autopsy after antemortem RT-QuIC testing was performed. Subsequently, we assess what effect RT-QuIC and new diagnostic criteria have on prion disease surveillance.

Methods

The primary research question was to determine whether variables, aside from having prion disease, affect CSF RT-QuIC results and the effect that RT-QuIC has on case ascertainment in the United States. This study meets Class III criteria for rating diagnostic accuracy studies in that it was performed in a cohort of patients with suspected prion disease who were referred for CSF testing, the diagnoses of which were ultimately verified by autopsy results (data available from Dryad, appendix, doi.org/10.5061/dryad.kwh70rz0g).

Patient and specimen selection

CSF specimens are sent to the NPDPSC by requesting clinicians when prion disease is suspected. US specimens received at the NPDPSC from May 2015 to October 2018 were included in this study. Only the individual’s first CSF specimen that produced a positive or negative RT-QuIC result was included in the analysis. The CSF specimen accession date is the day the specimen was received at the NPDPSC, which is usually within a week of specimen collection. Patient age was determined by the age at the time of CSF specimen accessioning by the NPDPSC. Individuals are referred for autopsy through the NPDPSC’s Autopsy Coordination Program by clinicians who suspect prion disease. Individuals were included in the autopsy subset if autopsy tissue was received between May 2015 and October 2018. Time variables were calculated, including time from illness onset to accessioning of the specimen, time from accessioning to death, and time from illness onset to death. Some requests did not include data on date of illness onset and were not included in related analyses.

Antemortem CSF testing

Total tau, 14-3-3, and second-generation RT-QuIC testing was performed on CSF specimens per standard of care in the NPDPSC’s Clinical Laboratory Improvement Amendments–licensed clinical laboratory. Total tau was measured by a quantitative ELISA (Life Technologies, Carlsbad, CA); 14-3-3 was evaluated qualitatively by Western blot with an anti-14-3-3 beta monoclonal antibody (Abcam, Cambridge, MA); and second-generation RT-QuIC was performed as previously described. These 3 tests were intended to be performed on all individuals’ CSF specimens. Because bloody specimens can yield falsely negative RT-QuIC results, overtly bloody specimens were excluded, but noncolorless (e.g., slightly bloody or icteric) specimens were tested. In the analyses, tau and 14-3-3 results were obtained from the same specimen as the RT-QuIC result.

Autopsy evaluation

Autopsy evaluation is the gold standard for prion disease diagnosis and was used as the reference standard in this study. Individuals with fixed or frozen autopsy tissue were included in the autopsy subgroup. Standard-of-care neuropathologic autopsy examination by a neuropathologist at the NPDPSC (M.L.C.) included hematoxylin and eosin staining and immunohistochemical examination, as well as proteinase K-resistant prion protein detection by Western blot. Individuals with only fixed tissue were not able to be genetically characterized but were qualitatively diagnosed with prion disease (i.e., prion disease not...
otherwise specified), and individuals with frozen tissue were
diagnosed to subtype as per commonly used criteria. Genetic
testing of the prion protein gene (PRNP) for mutations and
codon 129 polymorphism was conducted by University Hos-
pitals Cleveland Medical Center Department of Pathology.

**Statistical analysis**
Contingency table analyses were used to evaluate the de-
mographics among the study populations across RT-QuIC results. The p values were derived from the Fisher exact test (2-by-2 tables) or $\chi^2$ test. We used t tests to compare continuous variables. Multivariate binary logistic regressions were conducted to assess potential factors that affect the sensitivity and false negativity of RT-QuIC. Factors included sex, age as a continuous variable, and specimen color. In the assessment of sensitivity, an exploratory analysis was conducted among sCJD cases, with PRNP codon 129 polymorphism (MM, MV, VV) included as a factor. In the multivariate analysis of factors associated with false negativity of RT-QuIC, 14-3-3 results and tau levels (<500, 500–1,150, 1,151–2,499, and >2,499 pg/mL) were included. Annual and average annual incidence rates were calculated with the corresponding US census 2014 national projections for each year used as the denominator. These rates were age adjusted by the direct method using the year 2000 standard US population. The numbers of definite and probable cases from the NPDPSC were taken from 2016 to 2018 (all inclusive). The 2-sided type 1 error level was 0.05. SAS version 9.4 (SAS Institute Inc, Cary, NC) and IBM SPSS Statistics version 25 (IBM, Armonk, NY) were used in conducting analyses.

**Standard protocol approvals, registrations, and patient consents**
This study was approved by the University Hospitals Cleveland Medical Center Institutional Review Board.

**Data availability**
Anonymized data not published in this article are available on request to qualified investigators.

**Results**
During the collection period, 11,016 CSF specimen were received from 10,778 unique individuals. Of the 11,016 speci-
mens accessioned, 238 (2.2%) were excluded because they

### Table 1 Description of individuals' CSF specimens and demographic characteristics divided by RT-QuIC results (n = 10,498)

| Characteristic                  | Positive (n = 1,103) | Negative (n = 9,395) | p Value<sup>e</sup> |
|--------------------------------|---------------------|----------------------|---------------------|
| Age, y                         | 67.4 ± 9.4          | 64.7 ± 13.5          | <0.001              |
| Male, n (%)                    | 559 (50.9)          | 4,880 (52.8)         | 0.234               |
| Ethnicity, n (%)               |                     |                      | <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, n (%)                    |                     |                      | <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, n (%)         | 920 (83.4)          | 2,334 (24.8)         | <0.001              |

Abbreviation: RT-QuIC = second-generation real-time quaking-induced conversion.
<sup>a</sup> Values are mean ± SD for continuous variables and number (column percent) for categorical variables.
<sup>b</sup> Numbers may not sum to total due to missing data, and percentages may not sum to 100% due to rounding.
<sup>c</sup> The p value is for analysis of variance F test (continuous variables) or $\chi^2$ test (categorical variables).
were not the individual’s first specimen to be tested; 196 (1.8%) were excluded because they were too bloody for RT-QuIC testing; and 84 (0.8%) were excluded because the CSF volume was inadequate, the order was canceled before the test was performed, the results were indeterminate, or the results were not available for unknown reasons. The remaining 10,498 specimens from unique individuals were included in this study. Of the 10,498, 567 (5.4%) had autopsy tissue analyzed at the NPDPSC during the study period, 497 (87.7%) of which were positive for prion disease on neuropathologic examination.

Of the 10,498 RT-QuIC results, 1,103 (10.5%) were positive (table 1). The RT-QuIC–positive group had an older mean age (p < 0.001). Data on race and ethnicity were largely lacking, but in this limited dataset, non-Hispanic white individuals were more likely to be RT-QuIC positive than other ethnic groups (p < 0.001). Total tau concentration was higher (p < 0.001) and 14-3-3 was more likely to be positive (p < 0.001) in RT-QuIC–positive specimens than RT-QuIC–negative specimens. Both 14-3-3 and RT-QuIC were associated with significantly different tau concentrations, with RT-QuIC positivity having greater tau concentrations than 14-3-3 (figure 1).

Autopsy was performed on 567 referrals (table 2), of which 117 (20.6%) had a negative RT-QuIC result. However, 48 (41%) of these individuals were positive for prion disease by autopsy, indicating a false-negative RT-QuIC result. Of the 450 individuals with positive RT-QuIC results, 449 (99.8%) were positive for prion disease at autopsy. One specimen had a false-positive RT-QuIC result, the final diagnosis of which was multifactorial dementia (Alzheimer disease and vascular). The overall sensitivity and specificity of RT-QuIC of all autopsied cases for prion disease were 90.3% and 98.5%, respectively. The sensitivity of RT-QuIC was greatest in sCJD (92.9%) and genetic Creutzfeldt-Jakob disease (96.8%). No cases of fatal insomnia (sporadic or familial, n = 9) were detected by RT-QuIC. Individuals with prion disease who tested negative by RT-QuIC were younger and had a longer duration from illness onset to death, longer time from onset to CSF sample accession, and longer time from specimen accession to death. Sensitivity was high in all phases of the illness. Sensitivity was 83.3% (n = 8, 95% confidence interval [CI] 43.7–97.0) when time from disease onset to CSF collection was within 14 days, 91.9% (n = 41, 95% CI 78.7–97.2) when it was within 28 days, and 90.3% (n = 518, 95% CI 87.2–92.7) when CSF was collected ≥28 days after illness onset.

The autopsy sample included 439 individuals with sCJD (table 3). sCJD cases with falsely negative RT-QuIC results were younger, had lower CSF total tau levels, were less likely to be positive for 14-3-3, had a longer duration from illness onset to death, and had CSF specimens that were accessioned earlier before death compared to individuals whose specimens yielded positive RT-QuIC results. Codon 129 polymorphism did not correlate with the accuracy of RT-QuIC testing, but accuracy varied across molecular subtypes, with greatest sensitivity occurring in the most common forms of sporadic prion disease (sCJD MM1 and VV2, 96.3%), and MM2 and VV1 subtypes had a higher likelihood of producing false-negative RT-QuIC result.

Adverse events from performing the autopsy or standard-of-care CSF testing were not ascertained.

**Multivariate analyses of factors determining RT-QuIC positivity**

In the autopsy-confirmed prion disease sample (excluding the false RT-QuIC–positive case) (n = 497), a binary outcome of RT-QuIC positivity was modeled with logistic regression (table 4). Statistically significant factors were sex, with female individuals more likely to be RT-QuIC positive (odds ratio [OR] 2.522, 95% CI 1.260–5.043, p = 0.009), and age, with older individuals more likely to be positive (per 1-year increase, OR 1.115, 95% CI 1.075–1.157, p < 0.001). Specimen color was borderline significant, with noncolorless samples...
Table 2 Description of individuals with RT-QuIC testing who subsequently underwent autopsy (n = 566)²

| Characteristic            | True RT-QuIC positives (n = 449)² | False RT-QuIC negatives (n = 48)² | True RT-QuIC negatives (n = 69)² | p Valued |
|---------------------------|-----------------------------------|-----------------------------------|---------------------------------|----------|
| Age, y                    | 66.7 ± 8.3                        | 58.2 ± 9.8                        | 66.4 ± 10.5                     | <0.001   |
| Male, n (%)               | 236 (53)                          | 34 (71)                           | 38 (55)                         | 0.083    |
| Ethnicity, n (%)          |                                   |                                   |                                 | 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                   | 46 (95.8)                         | 67 (97.1)                         |                                 |          |
| Race, n (%)               |                                   |                                   |                                 | 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, n (%)    | 373 (83.1)                        | 18 (37.5)                         | 42 (60.9)                       | <0.001   |
| Mean time from illness onset to accession, median, d | 143.6 ± 203.3; 85 | 272.5 ± 746.1; 139 | 165.3 ± 353.2; 104 | 0.028 |
| Mean time from accession to death, median, d | 63.8 ± 101; 27 | 160.4 ± 208; 96 | 64.8 ± 122; 23 | <0.001 |
| Mean time from illness onset to death, median, d | 207.3 ± 248.2; 119 | 437.5 ± 870.1; 261 | 222.7 ± 339.8; 140 | <0.001 |
| Case diagnosis, n (%)     |                                   |                                   |                                 | <0.001   |
| Positivea                 | 449 (100)                         | 48 (100)                          | 0 (0)                           |          |
| Sporadic                  |                                   |                                   |                                 |          |
| sCJD                      | 408 (90.9)                        | 31 (64.6)                         |                                 |          |
| sFI                       | 0 (0.0)                           | 5 (10.4)                          |                                 |          |
| VPSPPr                    | 2 (0.5)                           | 1 (2.1)                           |                                 |          |
| Genetic                   |                                   |                                   |                                 |          |
| gCJDF                     | 30 (6.7)                          | 1 (2.1)                           |                                 |          |
| FFI                       | 0 (0.0)                           | 4 (8.3)                           |                                 |          |
| GSS diseaseg              | 1 (0.2)                           | 2 (4.2)                           |                                 |          |
| Negative                  | 0 (0.0)                           | 0 (0.0)                           | 69 (100.0)                      |          |

Abbreviations: FFI = fatal familial insomnia; gCJD = genetic Creutzfeldt-Jakob disease; GSS = Gerstmann-Sträussler-Scheinker; RT-QuIC = second-generation real-time quaking-induced conversion; sCJD = sporadic Creutzfeldt-Jakob disease; sFI = sporadic fatal insomnia; VPSPPr = variably protease sensitive prionopathy.

* One falsely positive RT-QuIC case (RT-QuIC+/autopsy−) was excluded from the table and the analyses.

+ Values are mean ± SD for continuous variables and number (column percent) for categorical variables.

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

* The p value is for t test (continuous variables) or χ² test (categorical variables).

a One case was included in which disease subtype is pending.

b gCJD includes the following mutations: E200K-129M (n = 18), E200K-129V (n = 3), V210I-129M (n = 7), A133V-129M (n = 1), V203I-129M (n = 1), and 2-OPRI-129M (n = 1).

c GSS includes the following mutations: P102L-129M (n = 2) and A117V-129V (n = 1).
| Characteristic                        | RT-QuIC positive (n = 408) | RT-QuIC negative (n = 31) | p Value |
|--------------------------------------|-----------------------------|---------------------------|---------|
| Age, y                               | 67.4 ± 8.4                  | 61.2 ± 9.3                | <0.001  |
| Male, n (%)                          | 214 (52.5)                  | 21 (68)                   | 0.1     |
| Ethnicity, n (%)                     |                             |                           | 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, n (%)                          |                             |                           | 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, n (%)               | 337 (82.6)                  | 14 (45.2)                 | <0.001  |
| Mean time from illness onset to accession, median, d | 143 ± 191; 88 | 156 ± 125; 126 | 0.732 |
| Mean time from accession to death, median, d | 65 ± 103; 27.5 | 149 ± 199; 91 | 0.026 |
| Mean time from illness onset to death, median, d | 208 ± 239; 122 | 308 ± 248; 248 | 0.034 |
| sCJD subtype, n (%)                  |                             |                           | <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, n (%)                |                             |                           | 0.472  |
| MM                                   | 239 (58.6)                  | 15 (48.4)                 |         |
| VV                                    | 63 (15.4)                   | 5 (16.1)                  |         |
| MV                                   | 106 (26.0)                  | 11 (35.5)                 |         |

Abbreviations: RT-QuIC = second-generation real-time quaking-induced conversion; sCJD = sporadic Creutzfeldt-Jakob disease.

* Values are mean ± SD for continuous variables and number (column percent) for categorical variables.

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

c The p value is for t test (continuous variables) or \( \chi^2 \) test (categorical variables).
less likely to be RT-QuIC positive (OR 0.349, 95% CI 0.113–1.077, p = 0.067).

We conducted a similar analysis on the autopsy-confirmed sCJD sample (n = 439), including PRNP codon 129 polymorphism as a categorical factor. Statistically significant factors were specimen color, with colorless samples more likely to have a positive RT-QuIC result (OR 3.55, 95% CI 1.054–11.99, p = 0.041), and age, with older individuals more likely to be positive (per 1-year increase, OR 1.010, 95% CI 1.050–1.151, p < 0.001). Sex was borderline significant, with female patients more likely to be positive (OR 2.14, 95% CI 0.948–4.82 p = 0.064). Codon 129 polymorphism was not significantly associated with RT-QuIC positivity in the sCJD sample.

Factors associated with prion disease in RT-QuIC-negative samples

A subsample of autopsied cases with negative RT-QuIC results (n = 117) were analyzed with logistic regression (table 5). Factors included in the multivariate model were sex, age, specimen color, 14-3-3, and tau. Statistically significant factors included 14-3-3 results, with 14-3-3–negative results more likely to be prion positive (OR 15.479, 95% CI 3.400–70.462, p < 0.001). The categorical tau variable was also significantly different (p = 0.003), with the higher tau categories having higher probability of being positive. Age was also significant, with older individuals being less likely to have prion disease (per 1-year increase, OR 0.946, 95% CI 0.906–0.988, p = 0.012).

To assess whether RT-QuIC positivity is associated with disease progression, cases with multiple specimens were examined post hoc. Multiple specimens that yielded different RT-QuIC results were received from 12 individuals. Of these, 8 initially had RT-QuIC–negative results that converted to positive results on the second specimen, 4 of which came to autopsy and were confirmed to be prion disease. Of the 12 individuals with multiple specimens, 4 went from RT-QuIC–positive to RT-QuIC–negative results. These cases were found to have reproducible (4 of 4 wells) low-amplitude maximum fluorescence (7%, 8%, 8%, and 23%) that was interpreted as positive. None of these cases had autopsy data.

**RT-QuIC and prion disease surveillance**

Given that multiple countries have modified diagnostic criteria for probable sCJD to include RT-QuIC–positive cases in individuals with a neuropsychiatric disorder, we examined what effect these changes would have on surveillance. With the assumption that RT-QuIC testing is ordered due to suspicion of possible prion disease and hence in individuals with a neuropsychiatric disorder, we reviewed all cases sent to the NPDSC from 2016 to 2018 (inclusive) to determine the number of definite prion disease cases (as determined by neuropathologic examination) and

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**Table 4** RT-QuIC positivity among autopsy-confirmed positives (n = 497)<sup>a</sup>

| Parameter                        | RT-QuIC positive result | p Value | Adjusted OR value | 95% CI        |
|----------------------------------|-------------------------|---------|-------------------|---------------|
| Sex, female                      | 2.522                   | 0.009   | 1.260–5.043       |               |
| Specimen color, not colorless    | 0.349                   | 0.067   | 0.113–1.077       |               |
| Age, per 1-y increase            | 1.115                   | <0.001  | 1.075–1.157       |               |

Abbreviations: CI = confidence interval; OR = odds ratio; RT-QuIC = real-time quaking-induced conversion.

<sup>a</sup> One false RT-QuIC positive (RT-QuIC+/autopsy−) was excluded from the analyses.

**Table 5** Prion positivity among RT-QuIC negatives (n = 117)

| Parameter                        | Autopsy positive result | p Value | Adjusted OR value | 95% CI        |
|----------------------------------|-------------------------|---------|-------------------|---------------|
| Sex, female                      | 0.623                   | 0.331   | 0.240–1.618       |               |
| Specimen color, colorless        | 0.268                   | 0.136   | 0.048–1.514       |               |
| Tau, 500–1,150 pg/mL             | 2.820                   | 0.108   | 0.795–9.999       |               |
| Tau, 1,151–2,499 pg/mL           | 9.650                   | 0.007   | 1.852–50.277      |               |
| Tau, >2,499 pg/mL                | 27.466                  | <0.001  | 4.702–160.435     |               |
| 14-3-3, negative                 | 15.479                  | <0.001  | 3.400–70.462      |               |
| Age, per 1-y increase            | 0.946                   | 0.012   | 0.906–0.988       |               |

Abbreviations: CI = confidence interval; OR = odds ratio; RT-QuIC = real-time quaking-induced conversion.
probable cases of prion disease using the new criteria. RT-QuIC-positive cases were included only if they did not undergo neuropathologic examination. During this time period, 763 cases of prion disease were ascertained through neuropathologic examination, and an additional 683 cases were ascertained that were RT-QuIC positive but did not proceed to autopsy (data available from Dryad, table e-1, doi.org/10.5061/dryad.kwh70rz0g). Including RT-QuIC-positive cases that did not undergo post-mortem examination increased laboratory-based prion disease ascertainment by 90% (figure 2). Including RT-QuIC-positive cases in laboratory-based surveillance statistics results in an average annual crude incidence of prion disease of 1.48 cases per 1 million individuals from 2016 to 2018 (age-adjusted incidence rate 1.17). However, without neuropathologic examination, the definitive etiologies of these cases are unknown.

Discussion

Aside from brain biopsy, RT-QuIC is the only disease-specific antemortem biomarker for diagnosing prion disease that directly detects prions. We describe RT-QuIC results, other laboratory results, and patient demographics in a consecutive cohort of >10,000 individuals. Prion diagnoses were definitively identified or excluded by autopsy in >5% of this cohort. Similar to other studies, RT-QuIC demonstrated high sensitivity (90.3%) and specificity (98.5%) for detecting prion disease. RT-QuIC sensitivity was high throughout the duration of disease, which is important for ascertainment of cases for surveillance purposes and identifying individuals for potential treatment trials. Due to the large sample size, we were able to confidently identify several factors that are associated with limiting the sensitivity of RT-QuIC. Some genetic prion diseases (i.e., fatal familial insomnia and Gerstmann-Sträussler-Scheinker) and atypical sporadic prion disease subtypes (i.e., sporadic fatal insomnia, variably protease sensitive prionopathy, sCJD VV1, and sCJD MM2) are more likely to have false-negative RT-QuIC results. The most parsimonious explanation for this finding is strain variability. Future studies are required to examine the effect that different recombinant prion protein substrates may have on RT-QuIC results in these prion strains. Additional factors that correlate with decreased RT-QuIC sensitivity include younger age, male sex, and possibly specimen color. Features frequently observed in atypical prion diseases such as young age and negative 14-3-3 results were more common in cases of prion disease with false-negative RT-QuIC results. A subset of cases with multiple specimens found that some initially RT-QuIC-negative individuals converted to a positive RT-QuIC result and were confirmed to have prion disease at autopsy. Conversely, some cases that demonstrated low-amplitude fluorescent signal that were initially interpreted as positive later converted to RT-QuIC negative, suggesting that low-amplitude signal, even if reproducible, may be seen in the absence of prion disease. Further follow-up of such cases is required to interpret these results.

There are several limitations to this study. The largest limitation is that final diagnoses are known only for cases that proceed to autopsy. Hence, this study may miss false-positive RT-QuIC cases that do not proceed to autopsy and may miss false-negative cases in which prion disease is deemed clinically unlikely after a negative RT-QuIC result. Cases that proceed
to autopsy through the NPDPSC Autopsy Program are likely to be prion disease cases, which biases the autopsy cohort. The number of low-incidence prion disease subtypes was limited in this study, and additional studies are needed to better identify the reliability of RT-QuIC across genetic prion disease mutations and atypical sporadic prion diseases. Race and ethnicity data were lacking in many of the individuals, making it difficult to interpret the relevance of non-Hispanic whites being more likely to be RT-QuIC positive. The finding that women with autopsy-confirmed prion disease had a higher likelihood of being RT-QuIC positive is intriguing but unexplained and requires more investigation as to whether there are strain differences between men and women. Likewise, the younger age of false-negative RT-QuIC cases may be due to younger atypical prion disease subtypes and strain variations, resulting in differences in the propensity to seed with RT-QuIC. Some referrals also did not have illness onset data, resulting in their exclusion from some analyses.

Counting RT-QuIC–positive individuals as cases of probable prion disease improves public health surveillance of human prion diseases. Some cases of prion disease do not proceed to autopsy, and the use of subjective interpretation of prion disease diagnosis, although cost-efficient, can be suboptimal due to inaccuracies in death certificate reporting. Including RT-QuIC–positive cases in surveillance statistics increases the ability to ascertain incidence in a reliable and objective manner, will likely improve death certificate data, and will ultimately improve epidemiologic studies of prion disease in countries using this diagnostic test. Future studies should examine how including neuropathologically confirmed cases, RT-QuIC–positive cases, and positive death certificate data in surveillance statistics affects prion disease surveillance. Postmortem neuropathologic examination is the gold standard for diagnosing prion disease and determining etiology, and it is crucial to detect possible novel forms of prion disease. This study demonstrates that unusual prion diseases are the most likely to go undetected with RT-QuIC alone. Because of the chronic wasting disease epidemic among cervids (e.g., deer, elk) in North America, neuropathologic examination is more important than ever to detect the possible emergence of novel prion diseases in humans.13,14

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| Aleksandra Wrona, MPH | Yale University, New Haven, CT                                 | Data collection and analysis, statistical analysis, creation of tables, revision of manuscript |
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| Mark L. Cohen, MD     | National Prion Disease Pathology Surveillance Center, Cleveland, OH | Data collection and analysis, revision of manuscript                            |
| Brian S. Appleby, MD  | National Prion Disease Pathology Surveillance Center, Cleveland, OH | Design and conceptualized study, analyzed the data, performed statistical analysis, drafted and revised the manuscript for intellectual content |
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