ORIGINAL ARTICLE

A Test for Creutzfeldt–Jakob Disease Using Nasal Brushings

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ABSTRACT

BACKGROUND

Definite diagnosis of sporadic Creutzfeldt–Jakob disease in living patients remains a challenge. A test that detects the specific marker for Creutzfeldt–Jakob disease, the prion protein (PrP^{CJD}), by means of real-time quaking-induced conversion (RT-QuIC) testing of cerebrospinal fluid has a sensitivity of 80 to 90% for the diagnosis of sporadic Creutzfeldt–Jakob disease. We have assessed the accuracy of RT-QuIC analysis of nasal brushings from olfactory epithelium in diagnosing sporadic Creutzfeldt–Jakob disease in living patients.

METHODS

We collected olfactory epithelium brushings and cerebrospinal fluid samples from patients with and patients without sporadic Creutzfeldt–Jakob disease and tested them using RT-QuIC, an ultrasensitive, multiwell plate–based fluorescence assay involving PrP^{CJD}-seeded polymerization of recombinant PrP into amyloid fibrils.

RESULTS

The RT-QuIC assays seeded with nasal brushings were positive in 30 of 31 patients with Creutzfeldt–Jakob disease (15 of 15 with definite sporadic Creutzfeldt–Jakob disease, 13 of 14 with probable sporadic Creutzfeldt–Jakob disease, and 2 of 2 with inherited Creutzfeldt–Jakob disease) but were negative in 43 of 43 patients without Creutzfeldt–Jakob disease, indicating a sensitivity of 97% (95% confidence interval [CI], 82 to 100) and specificity of 100% (95% CI, 90 to 100) for the detection of Creutzfeldt–Jakob disease. By comparison, testing of cerebrospinal fluid samples from the same group of patients had a sensitivity of 77% (95% CI, 57 to 89) and a specificity of 100% (95% CI, 90 to 100). Nasal brushings elicited stronger and faster RT-QuIC responses than cerebrospinal fluid ($P<0.001$ for the between-group comparison of strength of response). Individual brushings contained approximately 10^5 to 10^7 prion seeds, at concentrations several $\log_{10}$ greater than in cerebrospinal fluid.

CONCLUSIONS

In this preliminary study, RT-QuIC testing of olfactory epithelium samples obtained from nasal brushings was accurate in diagnosing Creutzfeldt–Jakob disease and indicated substantial prion seeding activity lining the nasal vault. (Funded by the Intramural Research Program of the National Institute of Allergy and Infectious Diseases and others.)

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Prion diseases, or transmissible spongiform encephalopathies, are fatal neurodegenerative disorders in humans and animals.[1,2] Prion diseases include Creutzfeldt–Jakob disease, the Gerstmann–Sträussler–Scheinker syndrome, and fatal familial insomnia in humans. The most common form of human prion disease is sporadic Creutzfeldt–Jakob disease, with an incidence of approximately 1 case per million persons per year worldwide.[3] Sporadic Creutzfeldt–Jakob disease is clinically heterogeneous and includes forms characterized by psychotic symptoms, depression, and behavioral and personality changes.[4,5] Possible or probable sporadic Creutzfeldt–Jakob disease is defined on the basis of clinical features, as well as periodic sharp and slow wave complexes on electroencephalograms, a positive 14-3-3 protein assay of cerebrospinal fluid samples, and altered signals on brain magnetic resonance images (MRI).[6] Definite diagnosis of sporadic Creutzfeldt–Jakob disease requires neuropathological or immunochemical detection of the prion protein (PrP^{CJD}) in brain tissue.[7] The heterogeneity of sporadic Creutzfeldt–Jakob disease phenotypes is influenced by the methionine (M)–valine (V) polymorphism at codon 129 of the prion protein gene (*PRNP*)[8] and the glycoform type (1 or 2) of PrP^{CJD}. PrP^{CJD} arises through the post-translational conformational conversion of the normal endogenous PrP (PrP^{C} or PrP^{Sen}) and accumulates preferentially in nervous tissue. PrP^{CJD} is also the main component of the infectious Creutzfeldt–Jakob disease agent (prion)[1,2,9-13] and propagates itself by seeding or templating the assembly of PrP^{C} into misfolded multimers that can take the form of amyloid fibrils.[2,10,11,13-15]

Although PrP^{CJD} is the only specific marker for Creutzfeldt–Jakob disease, it has been difficult to identify a PrP^{CJD} assay and a procedure for obtaining a tissue specimen that together are sufficiently sensitive, noninvasive, and practical for use in living patients. Recently, testing of cerebrospinal fluid with a new in vitro PrP^{CJD} amplification technology, designated real-time quaking-induced conversion (RT-QuIC), has shown considerable promise as a highly specific diagnostic test for sporadic Creutzfeldt–Jakob disease.[16,17] In the RT-QuIC assay, recombinant prion protein ($rPrP^{Sen}$) is mixed, or seeded, with a small amount of PrP^{CJD}, resulting in the formation of amyloid fibrils that are detected by thioflavin T (ThT) fluorescence.[17,18] This quantitative assay detects seeding activity in brain homogenate from humans with sporadic Creutzfeldt–Jakob disease that is diluted by a factor of more than 10^8.[18,19] Such diluted samples contain femtogram levels of protease-resistant PrP^{CJD}. Applications of RT-QuIC to panels of cerebrospinal fluid samples from patients with and patients without sporadic Creutzfeldt–Jakob disease have shown sensitivities for the detection of sporadic Creutzfeldt–Jakob disease (percent positive of the total number of patients with the disease) of 80 to 90% and specificities (percent negative of total number of controls without the disease) of 99 to 100%.[16,17] These studies suggest that RT-QuIC can achieve greater diagnostic performance than tests for surrogate markers of sporadic Creutzfeldt–Jakob disease in the cerebrospinal fluid.

Because RT-QuIC analysis of cerebrospinal fluid failed to identify 10 to 20% of patients with sporadic Creutzfeldt–Jakob disease, we sought to improve the sensitivity and practicality of diagnostic testing by applying the RT-QuIC technology to olfactory epithelium samples. PrP^{CJD} accumulates in the olfactory epithelium of patients with sporadic Creutzfeldt–Jakob disease, which suggests that analyses of olfactory mucosa–biopsy specimens might be diagnostic for patients with sporadic Creutzfeldt–Jakob disease.[20,21] However, only small, discrete units of tissue are obtained in olfactory mucosa biopsies, and, given the fact that the olfactory epithelium in the nasal vault is interspersed with PrP^{CJD}-negative respiratory mucosa,[20] analyses of olfactory mucosa for markers of Creutzfeldt–Jakob disease might provide false negative results if the biopsy misses the olfactory epithelium. In addition, surgical complications such as bleeding, infections, or traumatic injury can occur. Here we describe a much safer and less invasive nasal-brushing procedure that allows a gentle collection of olfactory mucosa from a wide surface area of olfactory epithelium. In this study, we assessed how well RT-QuIC analysis of olfactory mucosa brushings could discriminate patients with sporadic Creutzfeldt–Jakob disease from controls who did not have the disease.

METHODS

PATIENTS WITH CREUTZFELDT–JAKOB DISEASE AND CONTROLS

We used a variety of tests (Table 1) to classify 31 patients with rapidly progressive dementia who

were referred to our institutions by their treating physicians from various regions of Italy because of possible or probable Creutzfeldt–Jakob disease. Two of the patients were subsequently found to have inherited Creutzfeldt–Jakob disease resulting from E200K *PRNP* mutations. At the time of referral and during follow-up, all the patients were classified according to updated clinical diagnostic criteria.[5] Olfactory mucosa controls included 12 patients with other neurodegenerative disorders such as Alzheimer's disease or Parkinson's disease (8 women and 4 men; mean [±SD] age, 70.8±8.8 years; range, 48 to 82) and 31 persons without neurologic disorders (11 women and 20 men; mean age, 52.1±15.0 years; range, 24 to 81), who were, in most cases, referred to the ear, nose, and throat clinic for other purposes (Table 2).

STUDY OVERSIGHT

The study was approved by the ethics committee at Istituto Superiore di Sanità (Italy), which is recognized by the Office for Human Research Protections of the U.S. Department of Health and Human Services. Informed consent for participation in research was obtained in accordance with the Declaration of Helsinki and the Additional Protocol to the Convention on Human Rights and Biomedicine, concerning Biomedical Research. All the sampling of olfactory mucosa was performed after written informed consent was obtained from each patient or the patient's representative. The analyses of human specimens that were performed at the National Institute of Allergy and Infectious Diseases were performed under Exemption 11517 for the use of encoded samples from the National Institutes of Health Office of Human Subjects Research Protections.

BRAIN TISSUE

Brain-tissue specimens were obtained at autopsy from 15 patients with sporadic Creutzfeldt–Jakob disease and were processed for neuropathological examination and biochemical analyses. Control brain samples were provided by the National Institute for Biological Standards and Control, United Kingdom. Brain homogenate samples, 10% (weight:volume), were prepared[22] and serially diluted in 0.1% sodium dodecyl sulfate (SDS) in phosphate-buffered saline (PBS) containing 1×N2 medium supplement (GIBCO) (SDS–PBS–N2) before RT-QuIC analysis.

OLFACTORY MUCOSA AND CEREBROSPINAL FLUID SAMPLES

Olfactory mucosa or cerebrospinal fluid samples (>0.5 ml) were obtained from the patients with possible or probable Creutzfeldt–Jakob disease at the time of sampling, as well as from the patients with other neurologic disorders, including probable Alzheimer's disease[23] (5 olfactory mucosa and 13 cerebrospinal fluid samples), probable Parkinson's disease[24] (4 olfactory mucosa and 4 cerebrospinal fluid samples), probable progressive supranuclear palsy (1 olfactory mucosa and 1 cerebrospinal fluid sample), definite progressive supranuclear palsy[25] (1 olfactory mucosa and 1 cerebrospinal fluid sample), and paraneoplastic limbic encephalitis (1 olfactory mucosa and 1 cerebrospinal fluid sample) (Table 2). In five of these patients with other neurologic disorders, both olfactory mucosa and cerebrospinal fluid samples were obtained. Additional olfactory mucosa control samples were obtained from the 31 persons without neurologic disorders who had been admitted to the ear, nose, and throat clinic for other purposes. Cerebrospinal fluid control samples were purchased from Innovative Research or obtained from the Neuropathology Laboratory at Verona University Hospital. Two separate cerebrospinal fluid samples were obtained from 4 of the patients with Creutzfeldt–Jakob disease. All cerebrospinal fluid and olfactory mucosa samples were stored at −80°C from a time shortly after harvest until use in this assay.

PREPARATION OF OLFACTORY MUCOSA SAMPLES

Patients were not sedated when the olfactory mucosa samples were obtained. After administration of a local vasoconstrictor (1% epinephrine) with the use of a nasal tampon, a rigid fiberoptic rhinoscope, enveloped with a disposable sheath (Slide-On EndoSheath System, Medtronic Xomed) to prevent contamination, was inserted into the nasal cavity of the patient to locate the olfactory mucosa lining the nasal vault (Fig. S1A in the Supplementary Appendix, available with the full text of this article at NEJM.org). A sterile, disposable brush (Kito-Brush, Kaltek or Hobbs Medical) was then inserted alongside the fibroscope, gently rolled on the mucosal surface, withdrawn, and immersed in saline solution in a 15-ml conical centrifuge tube. Cellular material was dissociated from the brush by means of vortexing. The

Table 1. Demographic Characteristics, Clinical Profiles, Diagnostic Factors, and Real-Time Quaking-Induced Conversion (RT-QuIC) Analyses of Patients with Creutzfeldt–Jakob Disease.*

Patient No.	Age	Sex	Genotype at Codon 129†	Clinical Signs at Onset	CSF Analysis‡		Time between Clinical Onset and Lumbar Puncture	Time between Clinical Onset and OM Brushing	Typical MRI§	PSWCs on EEG	Disease Duration	Diagnosis at Time of OM Brushing	Final Diagnosis	RT-QuIC Results	
					Tau Level	14-3-3 Level								OM, 1:250	CSF‡
	yr				*pg/ml*		*mo*				*mo*				
1	77	Male	MM	Ataxia, visual hallucinations	1297; after 10-day interval, >2400	+; after 10-day interval, +	3.0	3.5	Yes	Yes	4.0	Probable sCJD	sCJD, MM type 1	+	+; +
2	50	Female	MM	Ataxia, behavioral changes	>2400	+	2.0	3.0	Yes	Yes	6.0	Probable sCJD	sCJD, MM type 1	+	+
3	68	Female	MV	Ataxia, dementia	>2400	+	1.0	1.0	Yes	No	16.0	Probable sCJD	sCJD, MV type 1	+	+
4	73	Male	MM	Depression	>2400	+	3.0	5.0	Yes	Yes	8.0	Probable sCJD	sCJD, MM type 1	+	+
5	64	Female	VV	Ataxia	>2400	+	1.0	1.0	Yes	Yes	4.0	Probable sCJD	sCJD,VV type 2	+	+
6	64	Female	MV	Depression, ataxia	892; after 2.5-mo interval, >2400	+; after 2.5-mo interval, +	5.0; 7.5	9.0	Yes	No	17.0	Probable sCJD	sCJD, MV type 2	+	+; +
7	66	Male	MM	Visual hallucinations	>2400	+	1.0	1.5	Yes	Yes	2.0	Probable sCJD	sCJD, MM type 1	+	–
8	68	Female	MM	Ataxia, visual hallucinations	>2400	+	3.0	3.5	Yes	Yes	4.0	Probable sCJD	sCJD, MM type 1	+	+
9	77	Male	MM	Ataxia	ND	+	1.0	1.5	No	No	2.5	Probable sCJD	sCJD, MM type 1	+	+
10	69	Male	MM	Apraxia, epileptic seizures	>2400	+	0.5	0.5	No	No	2.0	Probable sCJD	sCJD, MM type 1	+	–
11	75	Female	MM	Ataxia	ND	–	2.0	2.0	Yes	No	2.0	Probable sCJD	sCJD, MM type 1	+	+
12	29	Female	MM	Depression, choreic movements	>2400	+	2.0	3.0	Yes	No	12.0	Probable sCJD	Probable sCJD	+	–
13	65	Male	MV	Depression, ataxia, extrapyramidal signs	2297; after 5-mo interval, >2400	+; after 5-mo interval, +	10.0; 15.0	14.0	Yes	No	23.0¶	Probable sCJD	Probable sCJD	+	–; +
14	61	Male	MM	Ataxia, cortical blindness	>2400	+	2.0	2.0	Yes	Yes	9.0	Probable sCJD	Probable sCJD	+	+
15	68	Male	NA	Ataxia, dementia	>2400	+	2.5	4.0	Yes	No	4.0	Probable sCJD	sCJD, type 1	+	+
16	68	Female	MM	Visual hallucinations, ataxia	>2400	+	2.0	2.5	Yes	Yes	2.0	Probable sCJD	sCJD,MM type 1	+	+

17	76	Female	MV	Behavioral changes, ataxia	>2400	+	3.0	3.5	Yes	Yes	4.0	Probable sCJD	sCJD, MV type 1	+	+
18	73	Male	NA	Ataxia, dementia	>2400	+	6.0	7.0	No	No	11.0¶	Probable sCJD	Probable sCJD	+	+
19	46	Male	MV	Depression, mild ataxia	>2400	+	5.0	27.0	Yes	No	28.0	Probable sCJD	Probable sCJD	+	–
20	61	Male	MM	Behavioral changes, ataxia	>2400	+	2.0	3.0	Yes	Yes	6.0¶	Probable sCJD	Inherited CJD (E200K)	+	+
21	74	Female	MV	Ataxia, hallucinations	>2400	+	11.0	13.0	Yes	No	16.0¶	Probable sCJD	Probable sCJD	+	+
22	83	Male	MM	Dementia	>2400	+	2.0	2.0	Yes	Yes	4.0¶	Probable sCJD	Probable sCJD	+	+
23	68	Female	MV	Behavioral changes, dementia, extrapyramidal signs	1630; after 2-mo interval, 1845	+; after 2-mo interval, +	6.0	28.0	Yes	No	31.0¶	Probable sCJD	Probable sCJD	+	+; +
24	73	Female	VV	Depression, ataxia	>2400	+	4.0	6.0	Yes	Yes	8.0	Probable sCJD	Probable sCJD	+	+
25	78	Female	MM	Right hemiparesis, dysarthria, myoclonus	ND	–	1.0	1.5	No	No	4.0	Possible sCJD	Probable sCJD	+	+
26	61	Female	MM	Cerebellar signs, dementia	ND	+	6.0	11.0	Yes	No	13.0¶	Probable sCJD	Inherited CJD (E200K)	+	–
27	61	Male	MM	Extrapyramidal signs, apraxia	ND	+	2.0	2.0	No	No	2.0	Probable sCJD	Probable sCJD	+	+
28	54	Male	NA	Ataxia, dementia	>2400	+	1.5	2.0	Yes	Yes	2.0	Probable sCJD	sCJD, type 1	+	+
29	72	Male	MV	Dementia, extrapyramidal signs	>1300	+	13.0	16.0	Yes	No	18.0¶	Probable sCJD	Probable sCJD	–	–
30	66	Female	NA	Depression	>2400	+	3.0	3.0	Yes	Yes	4.0¶	Probable sCJD	Probable sCJD	+	–
31	65	Male	MV	Dementia, extrapyramidal signs	ND	–	9.0	10.0	No	No	11.0¶	Possible sCJD	Probable sCJD	+	NA

* CJD denotes Creutzfeldt–Jakob disease, CSF cerebrospinal fluid, EEG electroencephalogram, NA not available, ND not determined, OM olfactory mucosa, PSWC periodic sharp and slow wave complex, and sCJD sporadic CJD.
† Prion protein gene (*PRNP*) sequencing analysis was performed on genomic DNA extracted from blood cells to test for methionine (M)–valine (V) polymorphisms at codon 129.
‡ Four patients had two lumbar punctures each, and one patient had no CSF sample tested.
§ A brain MRI result was classified as typical on the basis of standardized criteria.[5]
¶ Patient is still alive.

brush was withdrawn, and particulate matter was pelleted for 20 minutes at 2000×*g* at 4°C and frozen at −80°C. The olfactory mucosa pellet samples were thawed and centrifuged for 10 minutes at 3220×*g* at 4°C. The residual supernatant was removed, and a disposable inoculating loop (Fisherbrand) was dipped into the pellet to transfer approximately 1 to 2 μl of the pellet into a tube containing 25 μl PBS. The latter tube was sonicated until the pellet was dispersed and further diluted, as specified, in SDS–PBS–N2.

RT-QUIC ANALYSIS

For spiking experiments, 2 μl of brain homogenate dilutions in SDS–PBS–N2 were added to 48 μl of olfactory mucosa pellet after the latter had been diluted, as described above, in SDS–PBS–N2. RT-QuIC reactions were performed as described previously[18] with 2 μl of the spiked olfactory mucosa dilution as a seed and full-length hamster PrP residues 23 to 231 as the $rPrP^{Sen}$ substrate. The total reaction volume was 100 μl. For analysis of endogenous seeding activities, 2 μl of diluted olfactory mucosa or brain homogenate or 20 μl of undiluted cerebrospinal fluid was used to seed the reactions. RT-QuIC reactions were subjected to cycles of shaking and rest at 42°C for 55 to 90 hours as described previously.[18] The criteria for discriminating positive versus negative RT-QuIC tests of cerebrospinal fluid and olfactory mucosa samples are explained in the Methods section in the Supplementary Appendix.

STATISTICAL ANALYSIS

Statistical comparisons of mean relative ThT fluorescence responses in samples from patients with and patients without Creutzfeldt–Jakob disease were performed with unpaired t-tests. Calculations of 95% confidence intervals for sensitivities and specificities were performed with the use of the Wilson procedure.[26]

RESULTS

RT-QUIC ANALYSIS OF OLFACTORY MUCOSA SAMPLES SPIKED WITH BRAIN TISSUE

Brushings of the nasal vault (Fig. S1A in the Supplementary Appendix) yielded samples containing clusters of olfactory neurons (Fig. S1B and S1C in the Supplementary Appendix) in addition to respiratory ciliated cells and a few granulocytes and monocytes (data not shown). Spiking of an olfactory mucosa sample from a patient without Creutzfeldt–Jakob disease with brain homogenate from a patient with sporadic Creutzfeldt–Jakob disease that was diluted by a factor of $4x10^{-7}$ and $4x10^{-8}$ (containing approximately 20 femtograms and approximately 2 femtograms of protease-resistant PrP^{CJD}, respectively) induced strong ThT fluorescence, with the more concentrated brain homogenate giving more rapid amplification kinetics (Fig. S2 in the Supplementary Appendix). The increased fluorescence indicated formation of amyloid fibrils by the initially monomeric $rPrP^{Sen}$. In the case of both of the brain homogenate dilutions, similar kinetics were also observed when the brain homogenates were diluted into the same buffer without any olfactory mucosa, indicating that olfactory mucosa components (diluted 1:250 overall) did not inhibit RT-QuIC.

ENDOGENOUS OLFACTORY MUCOSA SEEDING ACTIVITY

RT-QuIC dilution analysis of an olfactory mucosa brushing sample from a patient with sporadic Creutzfeldt–Jakob disease showed that all four replicate reactions that were seeded with 1:250 and 1:2500 dilutions of the olfactory mucosa pellet gave strong ThT positivity within 30 hours (Fig. S3 in the Supplementary Appendix). The 1:25,000 dilution was ThT-positive in two of four replicate wells within approximately 50 hours. In contrast, none of the same dilutions of an olfactory mucosa sample from patients without Creutzfeldt–Jakob disease gave positive reactions within 60 hours. These data indicated that the olfactory mucosa pellets from the patient with sporadic Creutzfeldt–Jakob disease had several $logs_{10}$ of RT-QuIC seeding activity (see also below).

We then tested blinded sets of samples comprising 74 olfactory mucosa samples from patients with definite sporadic Creutzfeldt–Jakob disease (15 samples), probable sporadic Creutzfeldt–Jakob disease (14), or inherited (E200K *PRNP* mutation) Creutzfeldt–Jakob disease (2) and from negative controls without Creutzfeldt–Jakob disease (43). Table 1 summarizes the demographic and clinical features of the patients with sporadic Creutzfeldt–Jakob disease, as well as the results of MRI, electroencephalography, and cerebrospinal fluid analyses. The patients with sporadic Creutzfeldt–Jakob disease had MM, MV, or VV at residue 129

Table 2. Results of RT-QuIC Assays of Olfactory Mucosa and Cerebrospinal Fluid Samples.*

Patients and Controls	Olfactory Mucosa	Cerebrospinal Fluid		
	Positive RT-QuIC Assay	Positive RT-QuIC Assay	Positive 14-3-3 Assay	Positive for Tau >2400 pg/ml
		number/total number		
Patients with CJD	30/31	23/30	28/31	23/25
MM genotype	14/14	11/14	12/14	10/10
MV genotype	8/9	6/8	8/9	6/8
VV genotype	2/2	2/2	2/2	2/2
PRNP genetic analysis not performed	4/4	3/4	4/4	4/4
Inherited CJD, E200K mutation	2/2	1/2	2/2	1/1
Controls				
Patients with other neurologic disorders†	0/12	0/20	3/17	0/17
Persons with no neurologic disorder‡	0/31	0/26	NA	NA

* Olfactory mucosa samples from 31 patients with CJD were tested with an RT-QuIC assay; cerebrospinal fluid samples were tested with an RT-QuIC assay (30 patients), 14-3-3 assay (31 patients), and tau assay (25 patients). Although 2 of the 31 patients had been categorized only as having "possible" sporadic CJD at the time of olfactory mucosa sampling, all these patients have since been designated as having "probable" or "definite" sporadic CJD. In the case of some patients, two cerebrospinal fluid samples were tested; however, only data from the sample obtained nearest in time to the olfactory mucosa collection are reflected in the numbers shown here. NA denotes not available.

† In the case of 5 patients with other neurologic disorders, both olfactory mucosa samples and cerebrospinal fluid samples were obtained.

‡ These persons were referred to the ear, nose, and throat clinic for other purposes.

of the PrP sequence and either the type 1 or type 2 PrP^{CJD}, according to subsequent assessment of brain tissue.[8]

As evaluated according to the criteria described in the Methods section in the Supplementary Appendix, positive RT-QuIC reactions were observed in 1:250 dilutions of olfactory mucosa samples from 15 of 15 patients with definite sporadic Creutzfeldt–Jakob disease, 13 of 14 with probable sporadic Creutzfeldt–Jakob disease, and 2 of 2 with inherited (E200K) Creutzfeldt–Jakob disease (Table 2). Individual RT-QuIC traces from the first 14 patients with sporadic Creutzfeldt–Jakob disease are shown in Figures S4A through S4N in the Supplementary Appendix, and traces averaged from all the sporadic and inherited (E200K) cases of Creutzfeldt–Jakob disease are shown in Figure 1A. In contrast, olfactory mucosa samples from all 43 control patients without Creutzfeldt–Jakob disease were RT-QuIC–negative at the same dilution (Fig. 1A and 1B and Table 2, and Fig. S4O and S4P in the Supplementary Appendix). The final average normalized ThT fluorescence readings at 50 hours from four replicate reactions in specimens from all the patients with sporadic Creutzfeldt–Jakob disease and the controls without Creutzfeldt–Jakob disease are shown in Figure 1B. The single patient with apparent Creutzfeldt–Jakob disease (Patient 29) for whom neither olfactory mucosa nor cerebrospinal fluid samples showed a positive RT-QuIC reaction (Fig. 1B and Table 1) received a diagnosis of probable Creutzfeldt–Jakob disease on the basis of established criteria and had tau and 14-3-3 protein levels that were typical of sporadic Creutzfeldt–Jakob disease. Overall, we obtained 30 positive RT-QuIC tests from 31 patients with apparent Creutzfeldt–Jakob disease, representing an estimated sensitivity of 97% (95% confidence interval [CI], 82 to 100) for distinguishing patients with Creutzfeldt–Jakob disease from those without the disease. If we considered only the definite cases of sporadic Creutzfeldt–Jakob disease, the estimated sensitivity would be 100%

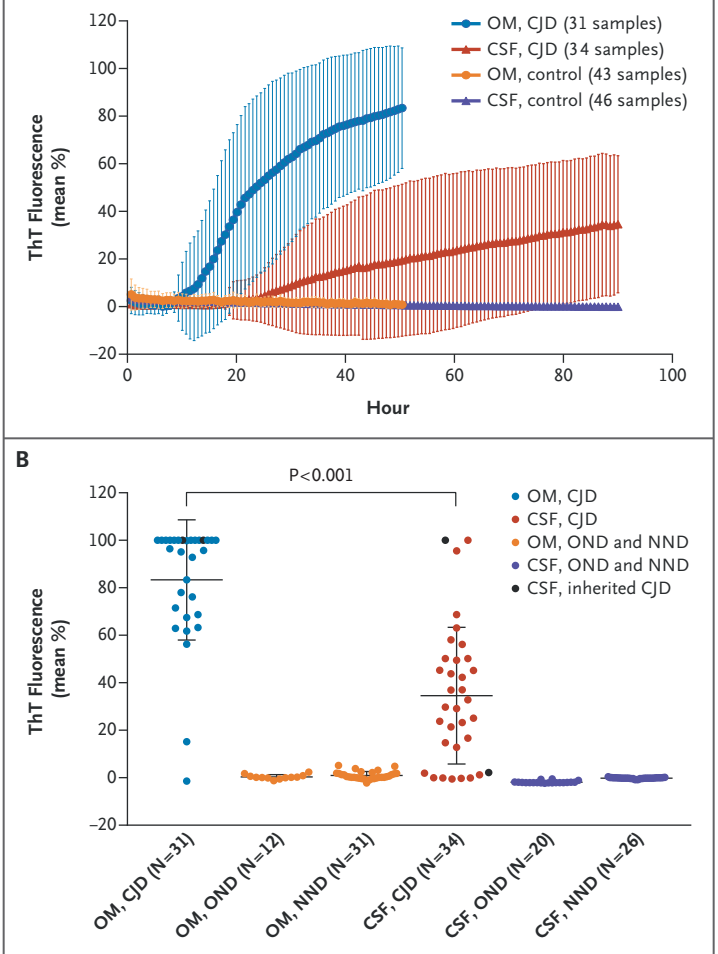

Figure 1. Results of Real-Time Quaking-Induced Conversion (RT-QuIC) Assays of Olfactory Mucosa (OM) and Cerebrospinal Fluid (CSF) Samples.

Panel A shows the average percent thioflavin T (ThT) fluorescence readings from four replicate reactions (normalized as described in the Methods section), determined in samples of OM and CSF from patients with possible, probable, or definite Creutzfeldt–Jakob disease and from controls without Creutzfeldt–Jakob disease. The means (thick lines) with standard deviations (thin lines) of those averages are shown as a function of RT-QuIC reaction time. Panel B shows the final average relative ThT fluorescence readings for each person with Creutzfeldt–Jakob disease (CJD) and for each control with either a neurologic disease other than Creutzfeldt–Jakob disease (other neurologic disease [OND]) or no neurologic disease (NND) at either 50 hours (OM samples) or 90 hours (CSF samples). Inherited CJD refers to patients with the E200K *PRNP* genetic mutation causing CJD. All OM and CSF samples obtained from patients with CJD and from controls are reflected in these data. The total number of CSF samples from patients with CJD (34) does not include a sample from one patient (Patient 31; CSF not tested) and includes results from two lumbar punctures each in four patients with CJD.

(95% CI, 75 to 100). The negative tests from all 43 control patients without Creutzfeldt–Jakob disease yielded a specificity of 100% (95% CI, 90 to 100).

RT-QUIC ANALYSIS OF CEREBROSPINAL FLUID

In contrast to the results of RT-QuIC analysis of olfactory mucosa samples, RT-QuIC analysis of cerebrospinal fluid samples had a sensitivity of 77% (95% CI, 57 to 89), with 23 RT-QuIC–positive reactions in 30 samples from patients with sporadic Creutzfeldt–Jakob disease, and a specificity of 100% (95% CI, 90 to 100), with 46 RT-QuIC–negative reactions in 46 samples from patients without Creutzfeldt–Jakob disease (Fig. 1 and Tables 1 and 2, and Fig. S4 in the Supplementary Appendix); these results were similar to those in previous studies.[16,17] For four of the patients with Creutzfeldt–Jakob disease, cerebrospinal fluid was obtained twice, as indicated in Table 1 and Figure 1; however, only the RT-QuIC results from the cerebrospinal fluid sample that was obtained closest in time to the olfactory mucosa collection are included in Table 2 and were used to calculate the RT-QuIC sensitivity. The olfactory mucosa pellets in 1:250 dilutions became positive much more rapidly than all but one of the undiluted cerebrospinal fluid samples from the same patients (Fig. 1A, and Fig. S4A through S4N in the Supplementary Appendix). In addition, the mean relative ThT fluorescence at our designated cutoff time (50 hours for olfactory mucosa and 90 hours for cerebrospinal fluid) was significantly higher in the olfactory mucosa samples than in the cerebrospinal fluid samples ($P<0.001$ with the use of an unpaired t-test) (Fig. 1B).

SEED CONCENTRATIONS IN SAMPLES FROM PATIENTS WITH SPORADIC CREUTZFELDT–JAKOB DISEASE

End-point dilution RT-QuIC analysis[18] of olfactory mucosa pellets (7 samples), brain-tissue samples (12), and cerebrospinal fluid samples (2) indicated that the concentrations of seeding doses resulting in 50% ThT-positive replicate reactions (SD_{50}) for these samples were 3.4 to 4.1 $\log_{10}$ SD_{50} per microliter of packed olfactory mucosa pellets, 7.2 to 8.95 $\log_{10}$ SD_{50} per milligram of brain tissue, and 0.2 to 0.5 (i.e., <0.1 $\log_{10}$) SD_{50} per microliter of cerebrospinal fluid. Thus, the seeding concentrations for olfactory mucosa were intermediate between those for cerebrospinal fluid and those for brain tissue. Considering that the olfactory mucosa brush specimens yielded packed

pellets of more than 50 μl, the total number of Creutzfeldt–Jakob disease prion seeds captured by individual brushings ranged from approximately 10^5 to 10^7.

DISCUSSION

The high sensitivity and specificity of RT-QuIC testing of nasal brushings in identifying patients with Creutzfeldt–Jakob disease indicate that this procedure has the potential to be useful for establishing a definitive diagnosis of Creutzfeldt–Jakob disease in living patients. Olfactory mucosa samples gave significantly stronger RT-QuIC responses than cerebrospinal fluid samples obtained from the same patients, and the RT-QuIC responses were obtained in less time (50 hours vs. 90 hours) (Fig. 1, and Fig. S4 in the Supplementary Appendix). Moreover, to date, the overall sensitivity of the olfactory mucosa–based RT-QuIC test for the diagnosis of sporadic Creutzfeldt–Jakob disease appears to be higher than that of the cerebrospinal fluid–based RT-QuIC test in the same patients. However, other studies have shown somewhat higher sensitivities for the RT-QuIC test of cerebrospinal fluid[16,17] than those obtained in this study. In any case, further validation of both the olfactory mucosa–based test and the cerebrospinal fluid–based test is needed to fully establish their relative diagnostic sensitivities and specificities in clinical settings and to determine how early seeding activity can be detected in the course of Creutzfeldt–Jakob disease. Furthermore, in six of seven patients with sporadic Creutzfeldt–Jakob disease in whom the RT-QuIC test of cerebrospinal fluid was negative but the RT-QuIC test of olfactory mucosa was positive, the lumbar puncture and olfactory mucosa brushing were performed at almost the same interval after disease onset, suggesting that the observed differences in prion seeding activity between these specimens were not due to differences in sampling time.

Although cerebrospinal fluid samples are likely to be obtained from most patients with rapidly progressing dementia to rule out other treatable disorders, RT-QuIC testing of olfactory mucosa may prove to be more accurate than RT-QuIC testing of cerebrospinal fluid for the detection of Creutzfeldt–Jakob disease. In our study, all the patients with suspected sporadic Creutzfeldt–Jakob disease fulfilled the diagnostic criteria for probable or possible sporadic Creutzfeldt–Jakob disease at the time of olfactory mucosa brushing, including the two patients with a negative family history of dementia who subsequently received a diagnosis of inherited (E200K) Creutzfeldt–Jakob disease.

In sporadic Creutzfeldt–Jakob disease, clinical variants are linked to the M/V polymorphism at PrP residue 129, combined with the PrP^{CJD} type 1 or 2. These molecular factors appear to influence PrP conversion and accumulation kinetics in vivo and in some in vitro reactions, such as protein misfolding cyclic amplification (PMCA).[27] However, with RT-QuIC reactions that use the hamster $rPrP^{Sen}$ substrate, each of the subtypes of sporadic Creutzfeldt–Jakob disease is readily detectable, as observed with RT-QuIC analysis of cerebrospinal fluid samples.[16] Although the molecular basis for the broad convertibility of this substrate is not yet clear, if it can be widely replicated it will provide the practical advantage of requiring only a single test with a single substrate to screen for the full spectrum of sporadic Creutzfeldt–Jakob disease phenotypes.

The high seeding activity of sporadic Creutzfeldt–Jakob disease in olfactory mucosa suggests that infectivity may be present as well, posing biosafety implications. The multiple $\log_{10}$ of prion seeds that we have detected in the nasal vault lining of patients with sporadic Creutzfeldt–Jakob disease raise the possibility that sporadic Creutzfeldt–Jakob disease prions could contaminate nasal discharges. Nasal and aerosol-borne transmission of prion diseases have been documented in animal models,[28-32] but there is no epidemiologic evidence for aerosol-borne transmission of sporadic Creutzfeldt–Jakob disease. Moreover, although prion infectivity and RT-QuIC seeding activity have been detected in nasal lavages from prion-infected hamsters,[33,34] no transmission was apparent in nonhuman primates injected with human spongiform encephalopathy nasal mucus.[35] However, medical instruments that come into contact with the olfactory mucosa of humans with Creutzfeldt–Jakob disease might become contaminated with prions, which poses the question of whether iatrogenic transmission is possible.[36,37] Therefore, further study of possible biohazards posed by prions in the olfactory mucosa is warranted.

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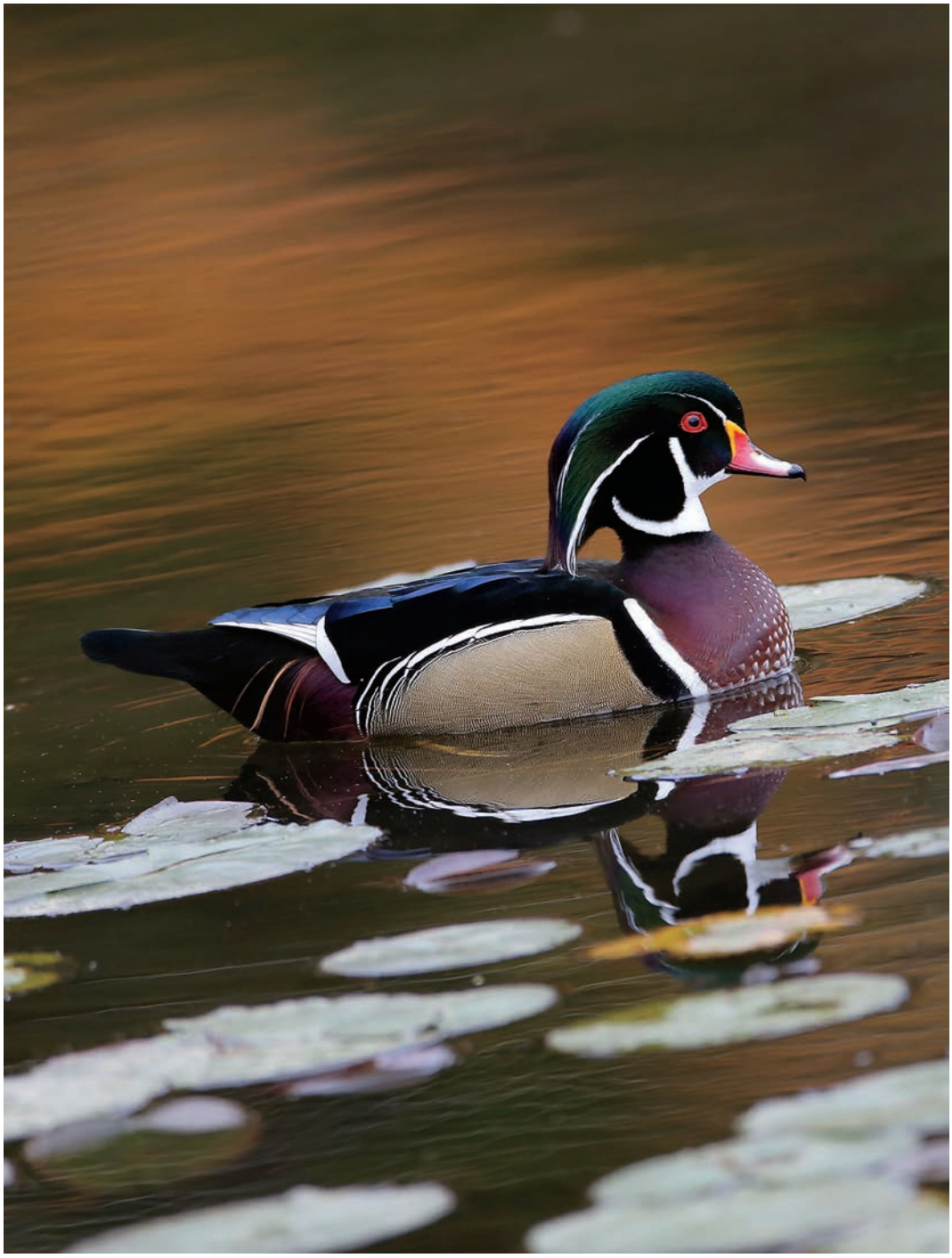
REFERENCES

1. Prusiner SB. Prions. Proc Natl Acad Sci U S A 1998;95:13363-83.
2. Caughey B, Baron GS, Chesebro B, Jeffrey M. Getting a grip on prions: oligomers, amyloids, and pathological membrane interactions. Annu Rev Biochem 2009;78:177-204.
3. Ladogana A, Puopolo M, Croes EA, et al. Mortality from Creutzfeldt-Jakob disease and related disorders in Europe, Australia, and Canada. Neurology 2005;64:1586-91.
4. Krasnianski A, Schulz-Schaeffer WJ, Kallenberg K, et al. Clinical findings and diagnostic tests in the MV2 subtype of sporadic CJD. Brain 2006;129:2288-96.
5. Puoti G, Bizzi A, Forloni G, Safar JG, Tagliavini F, Gambetti P. Sporadic human prion diseases: molecular insights and diagnosis. Lancet Neurol 2012;11:618-28. [Erratum, Lancet Neurol 2012;11:841.]
6. Zerr I, Kallenberg K, Summers DM, et al. Updated clinical diagnostic criteria for sporadic Creutzfeldt-Jakob disease. Brain 2009;132:2659-68. [Erratum, Brain 2012;135:1335.]
7. Budka H, Aguzzi A, Brown P, et al. Neuropathological diagnostic criteria for Creutzfeldt-Jakob disease (CJD) and other human spongiform encephalopathies (prion diseases). Brain Pathol 1995;5:459-66.
8. Parchi P, de Boni L, Saverioni D, et al. Consensus classification of human prion disease histotypes allows reliable identification of molecular subtypes: an inter-rater study among surveillance centres in Europe and USA. Acta Neuropathol 2012;124:517-29.
9. Deleault NR, Harris BT, Rees JR, Supattapone S. Formation of native prions from minimal components *in vitro.* Proc Natl Acad Sci U S A 2007;104:9741-6. [Erratum, Proc Natl Acad Sci U S A 2008;105:12636.]
10. Wang F, Wang X, Yuan CG, Ma J. Generating a prion with bacterially expressed recombinant prion protein. Science 2010;327:1132-5.
11. Kim JI, Cali I, Surewicz K, et al. Mammalian prions generated from bacterially expressed prion protein in the absence of any mammalian cofactors. J Biol Chem 2010;285:14083-7.
12. Legname G, Baskakov IV, Nguyen HO, et al. Synthetic mammalian prions. Science 2004;305:673-6.
13. Makarava N, Kovacs GG, Bocharova O, et al. Recombinant prion protein induces a new transmissible prion disease in wild-type animals. Acta Neuropathol 2010;119:177-87.
14. Kocisko DA, Come JH, Priola SA, et al. Cell-free formation of protease-resistant prion protein. Nature 1994;370:471-4.
15. Castilla J, Saá P, Hetz C, Soto C. In vitro generation of infectious scrapie prions. Cell 2005;121:195-206.
16. McGuire LI, Peden AH, Orrú CD, et al. Real time quaking-induced conversion analysis of cerebrospinal fluid in sporadic Creutzfeldt-Jakob disease. Ann Neurol 2012;72:278-85.
17. Atarashi R, Satoh K, Sano K, et al. Ultrasensitive human prion detection in cerebrospinal fluid by real-time quaking-induced conversion. Nat Med 2011;17:175-8.
18. 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.
19. Orrù CD, Hughson AG, Race B, Raymond GJ, Caughey B. Time course of prion seeding activity in cerebrospinal fluid of scrapie-infected hamsters after intratongue and intracerebral inoculations. J Clin Microbiol 2012;50:1464-6.
20. Zanusso G, Ferrari S, Cardone F, et al. Detection of pathologic prion protein in the olfactory epithelium in sporadic Creutzfeldt–Jakob disease. N Engl J Med 2003;348:711-9.
21. Tabaton M, Monaco S, Cordone MP, et al. Prion deposition in olfactory biopsy of sporadic Creutzfeldt-Jakob disease. Ann Neurol 2004;55:294-6.
22. Saá P, Castilla J, Soto C. Ultra-efficient replication of infectious prions by automated protein misfolding cyclic amplification. J Biol Chem 2006;281:35245-52.
23. Cummings JL, Dubois B, Molinuevo JL, Scheltens P. International Work Group criteria for the diagnosis of Alzheimer disease. Med Clin North Am 2013;97:363-8.
24. Hughes AJ, Daniel SE, Kilford L, Lees AJ. Accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinico-pathological study of 100 cases. J Neurol Neurosurg Psychiatry 1992;55:181-4.
25. Respondek G, Roeber S, Kretzschmar H, et al. Accuracy of the National Institute for Neurological Disorders and Stroke/Society for Progressive Supranuclear Palsy and neuroprotection and natural history in Parkinson plus syndromes criteria for the diagnosis of progressive supranuclear palsy. Mov Disord 2013;28:504-9.
26. Newcombe RG. Two-sided confidence intervals for the single proportion: comparison of seven methods. Stat Med 1998;17:857-72.
27. Jones M, Peden AH, Head MW, Ironside JW. The application of in vitro cell-free conversion systems to human prion diseases. Acta Neuropathol 2011;121:135-43.
28. Kincaid AE, Hudson KF, Richey MW, Bartz JC. Rapid transepithelial transport of prions following inhalation. J Virol 2012;86:12731-40.
29. Kincaid AE, Bartz JC. The nasal cavity is a route for prion infection in hamsters. J Virol 2007;81:4482-91.
30. Denkers ND, Hayes-Klug J, Anderson KR, et al. Aerosol transmission of chronic wasting disease in white-tailed deer. J Virol 2013;87:1890-2.
31. Haybaeck J, Heikenwalder M, Klevenz B, et al. Aerosols transmit prions to immunocompetent and immunodeficient mice. PLoS Pathog 2011;7(1):e1001257.
32. Denkers ND, Seelig DM, Telling GC, Hoover EA. Aerosol and nasal transmission of chronic wasting disease in cervidized mice. J Gen Virol 2010;91:1651-8.
33. Bessen RA, Shearin H, Martinka S, et al. Prion shedding from olfactory neurons into nasal secretions. PLoS Pathog 2010;6(4):e1000837.
34. Bessen RA, Wilham JM, Lowe D, et al. Accelerated shedding of prions following damage to the olfactory epithelium. J Virol 2012;86:1777-88.
35. Brown P, Gibbs CJ Jr, Rodgers-Johnson P, et al. Human spongiform encephalopathy: the National Institutes of Health series of 300 cases of experimentally

transmitted disease. Ann Neurol 1994;35:513-29.
36. Brown P, Brandel JP, Sato T, et al. Iatrogenic Creutzfeldt-Jakob disease, final assessment. Emerg Infect Dis 2012;18:901-7.
37. Flechsig E, Hegyi I, Enari M, Schwarz P, Collinge J, Weissmann C. Transmission of scrapie by steel-surface-bound prions. Mol Med 2001;7:679-84.



Male Wood Duck

Mac Greganti, M.D.