Supplementary Material and Methods

RT-QuIC Assay

RT-QuIC was conducted in a black, 96-well, optical-bottomed plate (Nunc, Roskilde, Denmark, 265301) on a FLUOstar plate reader (BMG Labtech, Offenburg, Germany). CSF samples (10 µL) were mixed with 10 µg recombinant hamster PrP23-231 ($rPrP^{C}$) in reaction buffer containing 10 mmol/L phosphate-buffered saline, 170 mM NaCl, 10 µM thioflavin T (ThT), 10 µmol/L EDTA, and 1 ng SDS. The final reaction volume was 100 µl. Each tested sample was duplicated. Each reaction contained blank (reaction buffer), negative (1 µL 10% brain homogenate from normal hamster), and positive controls (1 µL 10% brain homogenate from scrapie agent 263K-infected hamster). The working conditions were: temperature, 50ºC; vibration speed, 900 rpm; vibration/incubation time, 90/30 s; total reaction time, 90 h. ThT fluorescence (450 nm excitation and 480 nm emission) was automatically measured every 30 min as relative fluorescence units (rfu). The cutoff value was set as the average value of the negative controls plus 3 times the standard deviation (mean ± 3 SD). A sample was considered to be positive when the two parallel wells revealed positive reactive curves.

Supplementary Figure

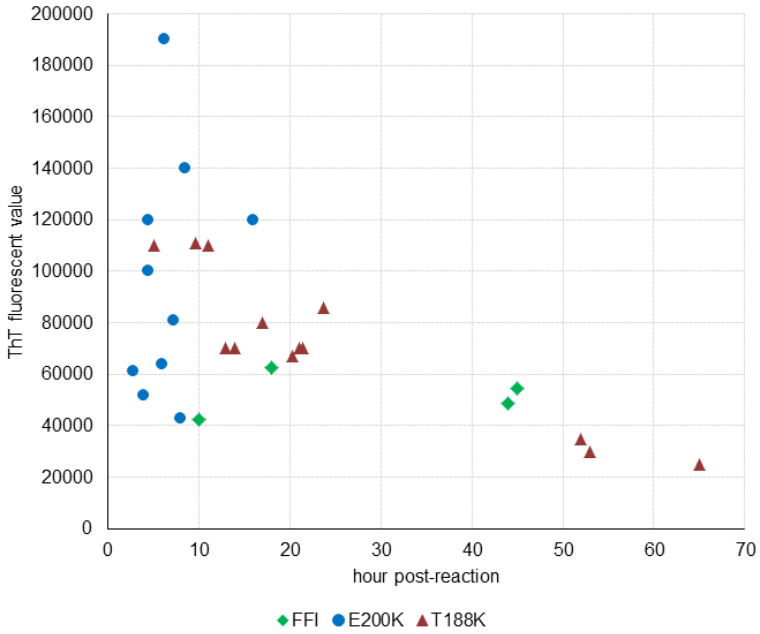

Fig. S1 Dot-plot of positive reactive results of CSF RT-QuIC assays in the D178N-FFI, T188K-fCJD, and E200K-fCJD groups. Thirteen T188K-fCJD samples (brown triangles), 10 E200K-fCJD samples (blue dots), and 4 D178N-FFI samples (green diamonds) showing positive reactions were included. X-axis, hours post-reaction; Y-axis, ThT fluorescence values.

Supplementary Table

Table S1 Features of CSF 14-3-3, EEG, MRI, and CSF RT-QuIC in patients with T188K-fCJD, D178N-FFI, and E200K-fCJD

	Case number	Gender (M/F)	CSF 14-3-3 positive	PSWCs on EEG	Abnormalities on MRI	RT-QuIC positive	Positive time post-reaction (h) (median)	Peak ThT value (rfu) (median)
T188K-fCJD	25	15/10	19 (76%)	4 (16%)	19 (76%)	13 (52%)	20.3	70000
D178N-FFI	24	10/14	5 (20.83%)	0 (0%)	2 (8.33%)	4 (16.7%)	31	51500
E200K-fCJD	16	7/9	16 (100%)	9 (56.25%)	15 (93.75%)	10 (62.5%)	6.1	90500