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MINIREVIEW

Clinical Laboratory Tests Used To Aid in Diagnosis of Human Prion Disease

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ABSTRACT Prion diseases are a group of rapidly progressive and always fatal neurodegenerative disorders caused by misfolded prion protein in the brain. Although autopsy remains the gold-standard diagnostic tool, antemortem laboratory testing can be performed to aid in the diagnosis of prion disease. This review is meant to help laboratory directors and physicians in their interpretation of test results. Laboratory assays to detect both nonspecific biomarkers of prion disease and prion-specific biomarkers can be used. The levels of nonspecific biomarkers in cerebrospinal fluid (CSF) are elevated when rapid neurodegeneration is occurring in the patient, and these markers include 14-3-3, tau, neuron-specific enolase, S100B, and alpha-synuclein. These markers have various sensitivities and specificities but are overall limited, as the levels of any of these analytes can be elevated in nonprion disease that is causing rapid damage of brain tissue. Prion-specific assays used in clinical laboratory testing are currently limited to two options. The first option is second-generation real-time quaking-induced conversion (RT-QuIC) performed on CSF, and the second option is Western blotting of a brain biopsy specimen used to detect protease-resistant prion protein. Although both tests have exquisite specificity, RT-QuIC has a sensitivity of 92 to 97.2% in symptomatic individuals, compared to the brain biopsy Western blot sensitivity of 20 to 60%. RT-QuIC was added to the Centers for Disease Control and Prevention's diagnostic criteria for prion disease in 2018. Other caveats of laboratory testing need to be considered, as sporadic, genetic, and acquired forms of prion disease have different clinical and laboratory presentations, and these caveats are discussed. Laboratory testing plays an important role in the diagnosis of prion disease, which is often challenging to diagnose.

KEYWORDS 14-3-3, CJD, prion, RT-QuIC, tau

Prion diseases are a group of invariably fatal, rapidly progressive neurodegenerative disorders. All mammals have prion protein (PrP), but the conformation is typically the normal cellular form of PrP (PrP^{C}). The pathology and transmission of prion disease are due to the misfolded prion protein, which is described as scrapie PrP (PrP^{Sc}) (1). In humans, prion diseases can be divided into sporadic (85 to 90%), genetic (10 to 15%), and acquired (<1%) etiologies. Sporadic Creutzfeldt-Jakob disease (sCJD), sporadic fatal insomnia (sFI), and variably protease-sensitive prionopathy (VPSPr) make up the sporadic prion diseases. Genetic prion diseases include genetic CJD (gCJD), fatal familial insomnia (FFI), and Gerstmann-Sträussler-Scheinker (GSS) disease. Acquired prion diseases occur by iatrogenic exposure (iatrogenic CJD [iCJD]) or ingestion (Kuru and variant CJD [vCJD]) (2–4). Diagnosing prion disease can be challenging, and laboratory testing can improve the clarity of the diagnostic picture. This review provides information to help laboratory directors and physicians accurately interpret laboratory test results.

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TYPES OF PRION DISEASE

The annual incidence of sCJD in the United States and throughout the world is about 1 to 2 cases per 1,000,000 individuals, but incidence increases with age. In the United States, about 1 in 6,000 deaths is attributable to prion disease (5). No known epidemiological link to a cause of sCJD has been identified, which is why it is described as "sporadic," and it is widely believed to be due to a posttranslational modification of PrP^C. Increased age and homozygosity at prion protein gene (*PRNP*) codon 129 are the only definitive risk factors. Typical symptomatology of sCJD includes dementia and myoclonus with progression over weeks to months to akinetic mutism and death. Gait disturbance and limb ataxia are the most common cerebellar signs, which are commonly present. However, not all sCJD cases yield a classic clinical presentation, and there can be prodromal constitutional symptoms as well as extrapyramidal signs, pyramidal signs, and visual disturbances (6–8). The mean age of onset of sCJD is 63 years, and the duration from the onset of symptoms until death is usually 4 to 6 months.

Genetic prion diseases are caused by mutations in *PRNP*. Approximately 40 different mutations have been reported, and they vary, sometimes significantly, in disease phenotype (age at onset, penetrance, duration, clinical symptoms, diagnostic test results, and neuropathology) (9). Familiarity with features of different genetic prion diseases and routinely performing genetic testing in possible prion cases are keys to obtaining an accurate diagnosis. Detection of *PRNP* gene mutations can be performed by sequencing DNA from patients' blood specimens or decedents' unfixed autopsy tissue. The characteristics of some common genetic prion diseases are summarized in Table 1.

Acquired prion diseases can be due to iatrogenic exposure (iCJD), zoonotic exposure (vCJD), or human exposure (kuru). iCJD has been primarily linked to cadaver-derived human growth hormone and dura mater grafts (2). However, corneal transplants, electroencephalogram (EEG) depth electrodes, and neurosurgical equipment have also been causes of iatrogenic CJD (2). vCJD has been transmitted via blood transfusion, but there is no epidemiological evidence of this route of transmission in sCJD or genetic prion diseases (2). As of 2012, iCJD has been identified in 228 cases due to dura mater grafts, 226 cases due to cadaveric human growth hormone, 4 cases due to contaminated neurosurgical equipment, 2 cases due to contaminated EEG depth electrodes, 2 cases due to corneal transplants, and 3 cases due to blood transfusion (vCJD only) (2). The current strategy to minimize infection involves identifying people at high risk for prion disease and preventing them from serving as tissue donors. Additionally, prion-specific sterilization techniques should be used on reusable neurosurgical equipment after potential exposure to prions. Patients with a family history of prion disease or who have received cadaveric dura mater grafts or human growth hormone are considered to be at high risk, as are those who have spent time in areas affected by bovine spongiform encephalopathy (BSE). Another method to limit the spread of secondary transmission of vCJD is the implementation of embargoes of biological specimens from areas where BSE is endemic (2). The iCJD incidence is currently very low due to contemporary infection control measures.

vCJD is transmitted by ingestion of infected meat contaminated with BSE. As of 2016, there have been a total of 231 recognized cases of vCJD worldwide, mostly from the United Kingdom and other European countries (10). There have been four reported cases of vCJD in the United States (11), and all four patients were likely exposed to the prions in another country: two in the United Kingdom, one Saudi Arabia, and one in either a middle eastern or eastern European country. vCJD is characterized by young age of onset and prolonged disease course. Typical EEG changes have not been found in vCJD as in sCJD (4), and cerebral spinal fluid (CSF) laboratory test results are typically unremarkable. Specific to vCJD is the pulvinar sign on brain magnetic resonance imaging (MRI), which is best visualized on fluid-attenuated inversion recovery (FLAIR) sequences as hyperintensity in the pulvinar nucleus of the thalamus.

The Kuru incidence has decreased to zero or near zero since the Fore-speaking

TABLE 1 Characteristics of genetic prion disease

Mutation	Mean age of onset (yr)	Penetrance (%)	Mean duration	Symptoms	Diagnostic test sensitivities (%)	References
E200K	60	54–100 (age dependent)	7 mo	Dementia, ataxia, vertical gaze palsy, peripheral neuropathy	CSF 14-3-3, 57–100; CSF total tau, 80–100; CSF RT-QuIC, 87; brain MRI, 50–88	50–56
V180I	77	~1	25 mo	Dementia, akinetic mutism, parkinsonism, myoclonus	CSF 14-3-3, 87; CSF total tau, 79–91; CSF RT-QuIC, 0–67.9; brain MRI, 99–100	57–61
V210I	59	~10	5 mo	Dementia, myoclonus	CSF 14-3-3, 90–100; CSF total tau, 100; CSF RT-QuIC, 100; brain MRI, 15–100	50, 52, 59, 62, 63
M232R	64	0.1	8 mo	Dementia, akinetic mutism, myoclonus, dystonia	CSF 14-3-3, 55–89; CSF total tau, 55–94; CSF RT-QuIC, 89; brain MRI, 85	58, 64–67
P102L	50	100	4.5 yr	GSS phenotype, ataxia, leg weakness, areflexia	CSF 14-3-3, 8–38; CSF total tau, 8–38; CSF RT-QuIC, 83–100; brain MRI, 25–30	52, 54, 64, 68
D178N-129M	51	93	18 mo	FFI phenotype, insomnia, dysautonomia, dementia	CSF 14-3-3, 13–50; CSF total tau, 7.1; CSF RT-QuIC, 0; brain MRI, 0	27, 35, 52, 69–71

peoples of Papua New Guinea ceased the practice of endocannibalism over a half-century ago. However, much has been learned from studying kuru, including the finding that acquired prions can persist asymptomatically after prion exposure for more than 5 decades before manifesting disease.

DIAGNOSTIC CRITERIA FOR PRION DISEASE

In 2018, the Centers for Disease Control and Prevention (CDC) modified its diagnostic criteria for sCJD, which is the most common form of prion disease (12). Definite sCJD requires neuropathological diagnosis, immunohistochemical confirmation, tissue Western blotting for proteinase-resistant PrP, or the presence of scrapie-associated fibrils. Probable sCJD criteria are fulfilled by (i) a neuropsychiatric disorder plus a positive real-time quaking-induced conversion (RT-QuIC) test or (ii) a rapidly progressive dementia with at least 2 of 4 of the following criteria: (i) myoclonus, (ii) visual or cerebellar signs, (iii) pyramidal or extrapyramidal signs, and (iv) akinetic mutism plus (i) periodic sharp-wave complexes (PSWCs) on EEG, (ii) a positive 14-3-3 test for the CSF in a patient with disease for less than 2 years, or (iii) diffusion-weighted imaging (DWI) or FLAIR abnormalities of the caudate and putamen and/or at least two cortical regions (excluding the frontal cortex), without evidence of an alternative diagnosis. Possible sCJD is defined as a progressive dementia with at least 2 of 4 of the following criteria: (i) myoclonus, (ii) visual or cerebellar signs, (iii) pyramidal/extrapyramidal signs, and (iv) akinetic mutism plus a duration of less than 2 years. Iatrogenic CJD is defined as a progressive cerebellar syndrome in a patient who received human cadaver-derived pituitary hormone or sCJD with a known high-risk exposure (see above). Genetic CJD is classified as definite/probable CJD plus a first-degree relative with definite/probable CJD or a neuropsychiatric disorder with a disease-specific *PRNP* mutation.

As described in the CDC's diagnostic criteria, the gold standard for definitive diagnosis of prion disease is histology of autopsy specimens; however, this review focuses on the utility of antemortem diagnosis of prion disease using laboratory testing, which fits the CDC's definition of "probable" prion disease. Obtaining a confident antemortem diagnosis of prion disease is important for infection control purposes, for excluding other difficult-to-diagnose but potentially treatable neurological diseases, and for helping to prepare the patient and loved ones for end-of-life care.

ANTEMORTEM DIAGNOSTIC TESTING

Antemortem clinical laboratory testing for the detection of markers of prion disease is currently limited to the use of CSF and brain biopsy specimens in symptomatic individuals. Heightened decontamination protocols are employed by clinical laboratories performing prion testing because prions are not inactivated completely using standard disinfection methods (13, 14). Blood testing, nasal brushings, and skin biopsy specimens have been used in research settings to identify prions, but these methods have not yet been validated for clinical use. Laboratory testing can be performed to identify nonspecific markers of rapid neurodegeneration that are typically present in prion diseases, and testing can be performed to specifically identify the presence of prions. The sensitivity, specificity, and potential utility of these assays depend on the assay itself (Table 2) and the type of prion disease being considered (Table 3). Currently, there is no clinical role for screening CSF of asymptomatic individuals for markers of prion disease. *PRNP* germ line sequencing can also be performed when considering genetic prion diseases. Nonlaboratory testing can also be useful in the diagnostic workup for suspected prion disease.

Laboratory testing for nonspecific biomarkers. Routine CSF laboratory analyses, including total protein, glucose, and cell counts, are typically within normal limits in cases of prion disease. Tests for nonspecific biomarkers of prion disease do not detect PrP^{Sc}, but these tests identify evidence of rapid neurodegeneration. Although rapid neurodegeneration is a hallmark of prion diseases, many other processes can also cause rapid neurodegeneration. Due to the low prevalence of prion disease and the accompanying low pretest probability, the positive predictive value for nonspecific biomark-

TABLE 2 Sensitivities and specificities of clinical laboratory tests

Test	Sensitivity (%)	Specificity (%)	Reference(s)
Nonspecific CSF biomarkers			
14-3-3	61–95	40–92	16–18
t-tau	87–90	67–75	16, 18
t-tau/p-tau ratio	94	86	18
NSE	80	83–92	18, 20–22
S100B			
sCJD	94	85	22
vCJD	78	76	26
gCJD	20–92		27
Alpha-synuclein	93–98	96–97	28–30
Specific biomarkers			
CSF RT-QuIC (2nd generation)	92–97.2	98.5–100	34, 35
Brain biopsy PrP^{Sc} Western blotting	20–60		39

ers is generally low. Markers of rapid neurodegeneration that are often elevated in prion diseases include the following: 14-3-3, tau, neuron-specific enolase (NSE), and S100B. Measurement of these analytes is performed using routine immunoassays, but different laboratories can use different methods to interrogate the same analyte. Commonly, enzyme-linked immunosorbent assays (ELISAs) are used to obtain a quantitative measurement of an analyte, and Western blotting can be used for qualitative analyses.

(i) 14-3-3. Measurement of protein 14-3-3 from CSF provides an indication of neuronal injury. Therefore, the level of 14-3-3 is elevated in many other diseases besides prion disease. 14-3-3 was the first CJD marker that was well described and popularized, and it remains a well-known marker of disease despite more newly described analytes having higher diagnostic utility than 14-3-3 (15). Hamlin et al. measured a 90% sensitivity and a 40% specificity for 14-3-3 (16). A limitation of 14-3-3 testing is that bloody CSF can lead to false-positive results (16).

Excluding patients with CJD, 14-3-3 testing was negative in 93% of patients with neurodegenerative disorders in one study; however, it was positive in some patients within inflammation, paraneoplastic/central nervous system (CNS) tumor, stroke, epileptic fit, psychiatric, and metabolic groups. 14-3-3 testing was also positive in 10.7% of patients with vascular dementia, 5.3% of patients with Lewy body dementia, and 5.8% of patients with Alzheimer's disease (AD) (17). A longitudinal multicenter study of 29,002 CSF samples collected over 10 years revealed a specificity of 92% and a sensitivity of 61 to 83% for 14-3-3 testing in detecting prion disease, but the positive predictive value was 47 to 83% (17). A comparative study of CSF biomarkers in CJD versus AD produced a sensitivity of 95% and a specificity of 78% for 14-3-3 (18). The pretest probability of diseases like AD is much higher than that of prion disease, and AD can present with a clinical picture that overlaps that of prion disease. Because of these reasons, the positive predictive value of 14-3-3 is limited.

(ii) Tau. Tau is involved in microtubule stabilization within neurons (19), and elevated levels of total tau (t-tau) have been found in the CSF of patients with AD, frontotemporal dementia, vascular dementia, dementia with Lewy bodies, traumatic brain injury, and stroke. However, t-tau elevation tends to be much greater in sCJD than in these other diseases. Furthermore, sCJD can be differentiated from other conditions based upon the phosphorylated tau (p-tau)-to-total tau (t-tau) ratio. In a study examining several neurodegenerative CSF biomarkers comparing CJD to AD, a low p-tau/t-tau ratio was the best biomarker for sCJD, with a specificity of 94% and a sensitivity of 86%. t-tau levels by themselves demonstrated a sensitivity of 90% and a specificity of 75% (18). Hamlin et al. measured a sensitivity of 87% and a specificity of 67% for t-tau (16). Limitations to consider include the finding that tau levels in the CSF may decrease with disease progression (16).

(iii) Neuron-specific enolase. Neuron-specific enolase (NSE) is found in neuronal cell bodies and axons as well as neuroendocrine cells. NSE is another maker of

TABLE 3 Utility of testing by type of prion disease suspected

Disease(s)	Most helpful premortem testing	Least helpful premortem testing
sCJD (~90% of prion disease)	DWI, MRI, and RT-QuIC have high sensitivity and specificity; MRI accuracy is best when interpreted by somebody with extensive expertise in prion MRI	EEG is often not abnormal until late in disease, if at all; 14-3-3 sensitivity and specificity are poor
gCJD (~10% of prion disease)	*PRNP* gene sequencing is diagnostic; MRI and RT-QuIC have high overall sensitivity and specificity, but accuracy may depend on specific mutation	EEG is often not abnormal until late in disease, if at all; 14-3-3 sensitivity and specificity are poor
FFI, GSS (<5% of prion disease)	*PRNP* gene sequencing is diagnostic in the setting of the appropriate clinical syndrome	CSF laboratory tests (RT-QuIC, 14-3-3, tau), EEG, and MRI are often within normal limits
vCJD (rare form of prion disease)	FLAIR MRI with characteristic pulvinar sign	EEG, 14-3-3, and RT-QuIC are frequently negative in vCJD

neurodegeneration and is elevated in patients with CJD compared to controls (20). The reported sensitivity is about 80%, with specificities ranging from 83 to 92% (18, 20–22). Both serum and CSF results have been studied, and some have found elevation in both CSF and serum, and others observed elevation only in the CSF (20, 23, 24). Due to the fact that NSE is a nonspecific marker of neurodegeneration, it is also elevated in the serum and CSF of patients with acute stroke (24), but NSE levels should return to normal levels with time after a stroke (25).

(iv) S100B. S100B is an astroglial protein. Increased S100B levels, reflecting increasing astrocytic activity, have been reported in sCJD cases, with a reported sensitivity of 94% and a specificity of 85% (22). The sensitivity and specificity of S100B in vCJD cases were 78% and 76%, respectively (26). Sensitivities in genetic prion diseases were more variable, ranging from 20% in FFI and 50% in GSS to 92% in gCJD cases (27).

(v) Alpha-synuclein. The exact reason for increased alpha-synuclein levels in sCJD is unclear, and some suggest that it is a marker of synaptic damage, while others suggest increased secretion of alpha-synuclein from sCJD-affected neurons (28). A chemiluminescence-based ELISA to detect alpha-synuclein has a reported sensitivity of 93 to 98% and a specificity of 96 to 97% in identifying sCJD (28–30). Good interlaboratory reproducibility has been reported (28). Limitations of CSF alpha-synuclein quantification includes potential false positivity in the presence of peripheral blood (28) and poor performance in FFI and GSS cases (30). Alpha-synuclein testing is not currently available for clinical diagnostic use in the United States.

(vi) Multianalyte analysis. The results of nonspecific analytes tend to trend together, but creating a singular interpretation using the results of multiple nonspecific analytes has the potential to improve diagnostic accuracy. Bahl et al. found that combined 14-3-3 and t-tau testing produced a specificity of 96% and a sensitivity of 84% in differentiating patients with sCJD from those with other neurological conditions. When combining a low p-tau/t-tau ratio and the presence of 14-3-3, the specificity was 96% and the sensitivity was 79%. Combining NSE and t-tau or the p-tau/t-tau ratio increased the specificity but decreased the sensitivity (18). Comparing 14-3-3 and t-tau testing, Hamlin et al. found t-tau to be the better test even if the results of the two tests were combined (16). In one study, the combination of the presence of 14-3-3 and either elevated t-tau levels or a low p-tau/t-tau ratio was most accurate for diagnosing sCJD (18). There is no generally accepted multianalyte panel of nonspecific markers that is used in the diagnostic workup of a case of suspected prion disease.

Laboratory testing using prion-specific assays. Laboratory tests that are specific for prion disease identify PrP^{Sc} in tissue either by demonstrating the inability of PrP^{Sc} to be degraded by proteolytic enzymes (e.g., proteinase K) or by propagating the misfolded conformation of PrP^{Sc} using PrP^{C} as the reagent substrate.

(i) Real-time quaking-induced conversion. Real-time quaking-induced conversion (RT-QuIC) is the first clinically available assay that specifically detects prions without requiring analysis of brain tissue. Because of the test's high specificity and because a lumbar puncture is much easier to perform than a brain biopsy, RT-QuIC has rapidly been adopted in clinical practice and diagnostic guidelines. This test was first offered clinically in the United States in 2015, and it was added to the CDC's diagnostic criteria in 2018.

RT-QuIC is a novel clinical assay that is unlike any other diagnostic test developed to date. The method leverages the autocatalytic nature of prions to aid in their detection. An elementary description of the method is as follows. A patient's specimen (e.g., CSF) that contains prions is used to seed reaction wells. The reaction wells contain reagents, including recombinant prion protein and thioflavin T (31). The mixture of the patient specimen and reagents is incubated and periodically shaken over the course of 60 h. During this time, the prion(s) in the original specimen can invoke a conformational change of the reagent prion protein *in vitro*, resulting in amyloid formation. The generation of amyloid during the reaction is identified by detecting an increase in the fluorescence of thioflavin T, followed by a characteristic decline in fluorescence, which

may be due to self-quenching (32). A characteristic fluorescence-time curve is interpreted as a positive result, which indicates that prions were present in the original specimen.

Currently, second-generation RT-QuIC is being used in clinical practice. The second-generation assay has been optimized by modifying reagents and incubation conditions (31). Direct comparison of first- and second-generation RT-QuIC showed an increase in sensitivity of 21% and a shortening of detection time of 2 days (33). A 2017 retrospective study of individuals from the United States showed that second-generation CSF RT-QuIC testing had a sensitivity of 92% and a specificity of 98.5%. A prospective study performed by the same group in 2017 showed a sensitivity of 95% and a specificity of 100% (34). Franceschini et al. reported a sensitivity of 97.2% and a specificity of 100% (35). Specimen types other than CSF have been used in research, but CSF is the only specimen type that is currently used for RT-QuIC diagnostic testing. RT-QuIC may also be able to differentiate the molecular subtypes of sCJD, but this is not yet used in clinical diagnostic testing (34, 36).

There are several sample qualities that may affect RT-QuIC test results. Samples remained stable over time and with repeated freezing and thawing (37). However, high CSF protein levels or white blood cell counts were found to lead to false-positive RT-QuIC results (36). Bloody CSF samples also interfere with the RT-QuIC assay, resulting in potentially false-negative results (34). It is recommended that the CSF sample to be tested contain fewer than 1,250 erythrocytes per μl (37). RT-QuIC's sensitivity is related to the type of prion strain that is causing disease, so not all prion diseases can be detected with high sensitivity (34). Also, very small amounts of prion may go undetected by RT-QuIC, but the validation of enrichment methods could potentially improve the limit of detection in the future (38).

(ii) Western blotting for PrP^{Sc}. Before RT-QuIC testing was available, the only prion-specific test for the antemortem diagnosis of prion disease was the use of a brain biopsy specimen, which was then analyzed for PrP^{Sc} by proteinase K treatment and subsequent Western blotting for prion protein. PrP^{C} is degraded by proteinase K treatment, but PrP^{Sc} remains intact and detectable by blotting. Although the test is specific for prion disease, the sensitivity can be low (20 to 60%) due to the spatial variability of prion deposition within the brain and the associated variability in obtaining a diagnostic specimen during collection. Additionally, identifying prion disease by brain biopsy creates exposure risks, and most importantly, it rarely changes the treatment plans (39). Therefore, brain biopsy is generally performed only for pathological characterization of suspected non-prion-related diseases. Although Western blotting is rarely used in antemortem diagnosis of prion disease, it is still commonly employed during autopsy to confirm the diagnosis and to characterize the molecular subtype of the prion strain (40).

Nucleic acid sequencing of the *PRNP* gene. Mutations and variations in the *PRNP* gene impact the predilection of an individual to develop prion disease. A common variant in *PRNP* is found at codon 129, which can encode methionine or valine. Codon 129 heterozygosity has been found to lower the risk of developing sCJD and some forms of acquired CJD, and although epidemiologically interesting, codon 129 polymorphism determination does not currently play any role in the diagnostic workup of a suspected case of prion disease. Full-gene sequencing of *PRNP* plays an important role in the identification of the etiology of individual cases of prion disease. Some cases of gCJD are clinically and histopathologically similar enough to sCJD in that differentiation between the two entities cannot be confidently discerned without sequencing of *PRNP*. Dozens of variants within *PRNP* have been identified and associated with mutated PrP, which is predisposed to misfolding and the development of gCJD, FFI, or GSS (9). Sequencing of *PRNP* of the proband can be performed using peripheral blood of the patient, or it can be performed on tissue from the decedent during autopsy as part of the full characterization of the prion disease. As with any genetic test, consultation with a genetic counselor before ordering the test should be considered.

Nonlaboratory testing. (i) Brain magnetic resonance imaging. Magnetic resonance imaging (MRI) is a helpful diagnostic tool when evaluating possible prion disease. Classic findings in sCJD include hyperintensity in both the caudate and putamen and/or at least two cortical regions (temporal, parietal, or occipital) on diffusion-weighted imaging (DWI) or fluid-attenuated inversion recovery (FLAIR) sequences (41), while in vCJD, hyperintensity in bilateral posterior thalami on FLAIR sequences is commonly seen (42). The utility of MRI in genetic prion diseases is more variable depending on the specific genetic mutations (Table 1). In many genetic prion diseases, only nonspecific findings such as atrophy are seen.

(ii) Electroencephalogram. Periodic sharp-wave complexes (PSWCs) seen upon electroencephalogram (EEG) were initially reported to be a typical finding in sCJD. However, it has been shown that PSWCs occur only during specific stages of sCJD and are of limited use in vCJD and gCJD, which limits their sensitivity (42). Moreover, PSWCs can also be seen in other neurodegenerative disorders as well as toxic/metabolic encephalopathies. Limited sensitivity and specificity as well as the increasing availability of other reliable testing methods make EEG less useful in diagnosing prion disease, but EEG remains crucial for ruling out other etiologies (e.g., seizures) of the presenting disease.

Timing of testing and utility of repeat testing. Through autopsy and surveillance studies, it is evident that the accuracy of diagnostic test results is impacted by the prion disease etiology, subtype, and time point of collection within the disease course. However, these variables are typically unknown in the midst of the diagnostic workup. Generally, CSF laboratory testing should be performed as soon as prion disease is suspected, and repeat testing is often not needed.

(i) Laboratory testing. In one study, CSF 14-3-3 levels were highest in cases with the shortest disease duration and reach maximum concentrations in the CSF midway through the disease course, followed by decreasing levels (43). Total tau concentrations in CSF showed a similar peak midway through the illness in another study (44). However, other studies have shown that total tau, S100B, and NSE increase with disease progression, with levels affected by codon 129 polymorphism (45). In our experience (our unpublished data), RT-QuIC sensitivity is mostly stable throughout the symptomatic course of disease, but in rare cases, retesting by RT-QuIC increases the diagnostic clarity.

CSF biomarkers of neurodegeneration must be interpreted in the context of the disease subtype, as factors besides disease progression affect results (Tables 1 to 3). The value of repeated CSF testing is largely unproven in the workup of possible prion disease, but if the clinical findings and laboratory findings are disparate, it is prudent to repeat testing.

(ii) Nonlaboratory testing. Diffusion restriction abnormalities occur on brain MRI early in the course of prion disease, but the abnormalities may be overlooked during routine interpretation (46). It may be useful to have a prion specialist interpret an MRI in cases of suspected prion disease (46). Diffusion restriction persists throughout the disease course and may progress to different brain areas over time (47). EEG findings change over the course of the disease, with classic PSWCs occurring in the middle to later stages of the illness, if present at all (48, 49).

CONCLUSION

Diagnosing prion disease can be clinically challenging, and the laboratory can play an important role in aiding in the diagnosis. Nonspecific markers of neurodegeneration that can be found in the CSF had been the mainstay of the diagnostic workup for decades, but recently, RT-QuIC has been implemented for clinical use, which is the first highly specific antemortem laboratory test than can be performed without a brain biopsy. Combining CSF laboratory findings and MRI findings is currently the best means to obtain an antemortem diagnosis of prion disease. Making an early and confident diagnosis of prion disease is helpful for infection control purposes and for patient care decision planning.

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REFERENCES

1. Prusiner SB. 1982. Novel proteinaceous infectious particles cause scrapie. Science 216:136–144. https://doi.org/10.1126/science.6801762.
2. Brown P, Brandel JP, Sato T, Nakamura Y, MacKenzie J, Will RG, Ladogana A, Pocchiari M, Leschek EW, Schonberger LB. 2012. Iatrogenic Creutzfeldt-Jakob disease, final assessment. Emerg Infect Dis 18:901–907. https://doi.org/10.3201/eid1806.120116.
3. Gajdusek DC, Zigas V. 1959. Kuru; clinical, pathological and epidemiological study of an acute progressive degenerative disease of the central nervous system among natives of the Eastern Highlands of New Guinea. Am J Med 26:442–469. https://doi.org/10.1016/0002-9343(59)90251-7.
4. Will RG, Ironside JW, Zeidler M, Cousens SN, Estibeiro K, Alperovitch A, Poser S, Pocchiari M, Hofman A, Smith PG. 1996. A new variant of Creutzfeldt-Jakob disease in the UK. Lancet 347:921–925. https://doi.org/10.1016/s0140-6736(96)91412-9.
5. Anonymous. 2019. Occurrence and transmission of Creutzfeldt-Jakob disease. Centers for Disease Control and Prevention, Atlanta, GA. https://www.cdc.gov/prions/cjd/occurrence-transmission.html. Accessed 5 July 2019.
6. Rabinovici GD, Wang PN, Levin J, Cook L, Pravdin M, Davis J, DeArmond SJ, Barbaro NM, Martindale J, Miller BL, Geschwind MD. 2006. First symptom in sporadic Creutzfeldt-Jakob disease. Neurology 66:286–287. https://doi.org/10.1212/01.wnl.0000196440.00297.67.
7. Wadsworth JD, Hill AF, Beck JA, Collinge J. 2003. Molecular and clinical classification of human prion disease. Br Med Bull 66:241–254. https://doi.org/10.1093/bmb/66.1.241.
8. Will RG, Matthews WB. 1984. A retrospective study of Creutzfeldt-Jakob disease in England and Wales 1970–79. I. Clinical features. J Neurol Neurosurg Psychiatry 47:134–140. https://doi.org/10.1136/jnnp.47.2.134.
9. Mastrianni JA. 2010. The genetics of prion diseases. Genet Med 12:187–195. https://doi.org/10.1097/GIM.0b013e3181cd7374.
10. Coulthart MB, Geschwind MD, Qureshi S, Phielipp N, Demarsh A, Abrams JY, Belay E, Gambetti P, Jansen GH, Lang AE, Schonberger LB. 2016. A case cluster of variant Creutzfeldt-Jakob disease linked to the Kingdom of Saudi Arabia. Brain 139:2609–2616. https://doi.org/10.1093/brain/aww206.
11. Anonymous. 2019. Tables of cases examined. Case Western Reserve University, Cleveland, OH. https://case.edu/medicine/pathology/divisions/national-prion-disease-pathology-surveillance-center/resources-professionals/tables-cases-examined-0. Accessed 5 July 2019.
12. Anonymous. 2019. CDC's diagnostic criteria for Creutzfeldt-Jakob Disease (CJD), 2018. Centers for Disease Control and Prevention, Atlanta, GA. https://www.cdc.gov/prions/cjd/diagnostic-criteria.html. Accessed 5 July 2019.
13. Anonymous. 2000. WHO infection control guidelines for transmissible spongiform encephalopathies. Report of a WHO consultation, Geneva, Switzerland, 23–26 March 1999. World Health Organization, Geneva, Switzerland. https://www.who.int/csr/resources/publications/bse/whocdscsraph2003.pdf.
14. Anonymous. 2009. Biosafety in microbiological and biomedical laboratories, 5th ed. US Department of Health and Human Services, Washington, DC. https://www.cdc.gov/labs/pdf/CDC-BiosafetyMicrobiologicalBiomedicalLaboratories-2009-P.PDF.
15. Hsich G, Kenney K, Gibbs CJ, Lee KH, Harrington MG. 1996. The 14-3-3 brain protein in cerebrospinal fluid as a marker for transmissible spongiform encephalopathies. N Engl J Med 335:924–930. https://doi.org/10.1056/NEJM199609263351303.
16. Hamlin C, Puoti G, Berri S, Sting E, Harris C, Cohen M, Spear C, Bizzi A, Debanne SM, Rowland DY. 2012. A comparison of tau and 14-3-3 protein in the diagnosis of Creutzfeldt-Jakob disease. Neurology 79:547–552. https://doi.org/10.1212/WNL.0b013e318263565f.
17. Stoeck K, Sanchez-Juan P, Gawinecka J, Green A, Ladogana A, Pocchiari M, Sanchez-Valle R, Mitrova E, Sklaviadis T, Kulczycki J, Slivarichova D, Saiz A, Calero M, Knight R, Aguzzi A, Laplanche JL, Peoc'h K, Schelzke G, Karch A, van Duijn CM, Zerr I. 2012. Cerebrospinal fluid biomarker supported diagnosis of Creutzfeldt-Jakob disease and rapid dementias: a longitudinal multicentre study over 10 years. Brain 135:3051–3061. https://doi.org/10.1093/brain/aws238.
18. Bahl JM, Heegaard NH, Falkenhorst G, Laursen H, Hogenhaven H, Molbak K, Jespersgaard C, Hougs L, Waldemar G, Johannsen P, Christiansen M. 2009. The diagnostic efficiency of biomarkers in sporadic Creutzfeldt-Jakob disease compared to Alzheimer's disease. Neurobiol Aging 30:1834–1841. https://doi.org/10.1016/j.neurobiolaging.2008.01.013.
19. Lee G, Leugers CJ. 2012. Tau and tauopathies. Prog Mol Biol Transl Sci 107:263–293. https://doi.org/10.1016/B978-0-12-385883-2.00004-7.
20. Zerr I, Bodemer M, Racker S, Grosche S, Poser S, Kretzschmar HA, Weber T. 1995. Cerebrospinal fluid concentration of neuron-specific enolase in diagnosis of Creutzfeldt-Jakob disease. Lancet 345:1609–1610. https://doi.org/10.1016/s0140-6736(95)90118-3.
21. Aksamit AJ, Jr, Preissner CM, Homburger HA. 2001. Quantitation of 14-3-3 and neuron-specific enolase proteins in CSF in Creutzfeldt-Jakob disease. Neurology 57:728–730. https://doi.org/10.1212/WNL.57.4.728.
22. Beaudry P, Cohen P, Brandel JP, Delasnerie-Laupretre N, Richard S, Launay JM, Laplanche JL. 1999. 14-3-3 protein, neuron-specific enolase, and S-100 protein in cerebrospinal fluid of patients with Creutzfeldt-Jakob disease. Dement Geriatr Cogn Disord 10:40–46. https://doi.org/10.1159/000017095.
23. Kohira I, Tsuji T, Ishizu H, Takao Y, Wake A, Abe K, Kuroda S. 2000. Elevation of neuron-specific enolase in serum and cerebrospinal fluid of early stage Creutzfeldt-Jakob disease. Acta Neurol Scand 102:385–387. https://doi.org/10.1034/j.1600-0404.2000.102006385.x.
24. Wakayama Y, Shibuya S, Kawase J, Sagawa F, Hashizume Y. 1987. High neuron-specific enolase level of cerebrospinal fluid in the early stage of Creutzfeldt-Jakob disease. Klin Wochenschr 65:798–801. https://doi.org/10.1007/bf01743256.
25. Kropp S, Zerr I, Schulz-Schaeffer WJ, Riedemann C, Bodemer M, Laske C, Kretzschmar HA, Poser S. 1999. Increase of neuron-specific enolase in patients with Creutzfeldt-Jakob disease. Neurosci Lett 261:124–126. https://doi.org/10.1016/s0304-3940(98)00992-6.
26. Green AJ, Thompson EJ, Stewart GE, Zeidler M, McKenzie JM, MacLeod MA, Ironside JW, Will RG, Knight RS. 2001. Use of 14-3-3 and other brain-specific proteins in CSF in the diagnosis of variant Creutzfeldt-Jakob disease. J Neurol Neurosurg Psychiatry 70:744–748. https://doi.org/10.1136/jnnp.70.6.744.
27. Ladogana A, Sanchez-Juan P, Mitrova E, Green A, Cuadrado-Corrales N, Sanchez-Valle R, Koscova S, Aguzzi A, Sklaviadis T, Kulczycki J, Gawinecka J, Saiz A, Calero M, van Duijn CM, Pocchiari M, Knight R, Zerr I. 2009. Cerebrospinal fluid biomarkers in human genetic transmissible spongiform encephalopathies. J Neurol 256:1620–1628. https://doi.org/10.1007/s00415-009-5163-x.
28. Llorens F, Kruse N, Karch A, Schmitz M, Zafar S, Gotzmann N, Sun T, Kochy S, Knipper T, Cramm M, Golanska E, Sikorska B, Liberski PP, Sanchez-Valle R, Fischer A, Mollenhauer B, Zerr I. 2018. Validation of alpha-synuclein as a CSF biomarker for sporadic Creutzfeldt-Jakob disease. Mol Neurobiol 55:2249–2257. https://doi.org/10.1007/s12035-017-0479-5.
29. Kruse N, Heslegrave A, Gupta V, Foiani M, Villar-Pique A, Schmitz M,

Lehmann S, Teunissen C, Blennow K, Zetterberg H, Mollenhauer B, Zerr I, Llorens F. 2018. Interlaboratory validation of cerebrospinal fluid alpha-synuclein quantification in the diagnosis of sporadic Creutzfeldt-Jakob disease. Alzheimers Dement (Amst) 10:461–470. https://doi.org/10.1016/j.dadm.2018.06.005.
30. Llorens F, Kruse N, Schmitz M, Gotzmann N, Golanska E, Thune K, Zejneli O, Kanata E, Knipper T, Cramm M, Lange P, Zafar S, Sikorska B, Liberski PP, Mitrova E, Varges D, Schmidt C, Sklaviadis T, Mollenhauer B, Zerr I. 2017. Evaluation of alpha-synuclein as a novel cerebrospinal fluid biomarker in different forms of prion diseases. Alzheimers Dement 13:710–719. https://doi.org/10.1016/j.jalz.2016.09.013.
31. Orru CD, Groveman BR, Hughson AG, Zanusso G, Coulthart MB, Caughey B. 2015. Rapid and sensitive RT-QuIC detection of human Creutzfeldt-Jakob disease using cerebrospinal fluid. mBio 6:e02451-14. https://doi.org/10.1128/mBio.02451-14.
32. Lindberg DJ, Wenger A, Sundin E, Wesen E, Westerlund F, Esbjorner EK. 2017. Binding of thioflavin-T to amyloid fibrils leads to fluorescence self-quenching and fibril compaction. Biochemistry 56:2170–2174. https://doi.org/10.1021/acs.biochem.7b00035.
33. Groveman BR, Orru CD, Hughson AG, Bongianni M, Fiorini M, Imperiale D, Ladogana A, Pocchiari M, Zanusso G, Caughey B. 2017. Extended and direct evaluation of RT-QuIC assays for Creutzfeldt-Jakob disease diagnosis. Ann Clin Transl Neurol 4:139–144. https://doi.org/10.1002/acn3.378.
34. Foutz A, Appleby BS, Hamlin C, Liu X, Yang S, Cohen Y, Chen W, Blevins J, Fausett C, Wang H, Gambetti P, Zhang S, Hughson A, Tatsuoka C, Schonberger LB, Cohen ML, Caughey B, Safar JG. 2017. Diagnostic and prognostic value of human prion detection in cerebrospinal fluid. Ann Neurol 81:79–92. https://doi.org/10.1002/ana.24833.
35. Franceschini A, Baiardi S, Hughson AG, McKenzie N, Moda F, Rossi M, Capellari S, Green A, Giaccone G, Caughey B, Parchi P. 2017. High diagnostic value of second generation CSF RT-QuIC across the wide spectrum of CJD prions. Sci Rep 7:10655. https://doi.org/10.1038/s41598-017-10922-w.
36. Green AJE. 2019. RT-QuIC: a new test for sporadic CJD. Pract Neurol 19:49–55. https://doi.org/10.1136/practneurol-2018-001935.
37. Cramm M, Schmitz M, Karch A, Mitrova E, Kuhn F, Schroeder B, Raeber A, Varges D, Kim YS, Satoh K, Collins S, Zerr I. 2016. Stability and reproducibility underscore utility of RT-QuIC for diagnosis of Creutzfeldt-Jakob disease. Mol Neurobiol 53:1896–1904. https://doi.org/10.1007/s12035-015-9133-2.
38. Denkers ND, Henderson DM, Mathiason CK, Hoover EA. 2016. Enhanced prion detection in biological samples by magnetic particle extraction and real-time quaking-induced conversion. J Gen Virol 97:2023–2029. https://doi.org/10.1099/jgv.0.000515.
39. Schuette AJ, Taub JS, Hadjipanayis CG, Olson JJ. 2010. Open biopsy in patients with acute progressive neurologic decline and absence of mass lesion. Neurology 75:419–424. https://doi.org/10.1212/WNL.0b013e3181eb5889.
40. Parchi P, Castellani R, Capellari S, Ghetti B, Young K, Chen SG, Farlow M, Dickson DW, Sima AA, Trojanowski JQ, Petersen RB, Gambetti P. 1996. Molecular basis of phenotypic variability in sporadic Creutzfeldt-Jakob disease. Ann Neurol 39:767–778. https://doi.org/10.1002/ana.410390613.
41. Zerr I, Kallenberg K, Summers DM, Romero C, Taratuto A, Heinemann U, Breithaupt M, Varges D, Meissner B, Ladogana A, Schuur M, Haik S, Collins SJ, Jansen GH, Stokin GB, Pimentel J, Hewer E, Collie D, Smith P, Roberts H, Brandel JP, van Duijn C, Pocchiari M, Begue C, Cras P, Will RG, Sanchez-Juan P. 2009. Updated clinical diagnostic criteria for sporadic Creutzfeldt-Jakob disease. Brain 132:2659–2668. https://doi.org/10.1093/brain/awp191.
42. Mackenzie G, Will R. 2017. Creutzfeldt-Jakob disease: recent developments. F1000Res 6:2053. https://doi.org/10.12688/f1000research.12681.1.
43. Gmitterova K, Heinemann U, Bodemer M, Krasnianski A, Meissner B, Kretzschmar HA, Zerr I. 2009. 14-3-3 CSF levels in sporadic Creutzfeldt-Jakob disease differ across molecular subtypes. Neurobiol Aging 30:1842–1850. https://doi.org/10.1016/j.neurobiolaging.2008.01.007.
44. Satoh K, Shirabe S, Tsujino A, Eguchi H, Motomura M, Honda H, Tomita I, Satoh A, Tsujihata M, Matsuo H, Nakagawa M, Eguchi K. 2007. Total tau protein in cerebrospinal fluid and diffusion-weighted MRI as an early diagnostic marker for Creutzfeldt-Jakob disease. Dement Geriatr Cogn Disord 24:207–212. https://doi.org/10.1159/000107082.
45. Sanchez-Juan P, Sanchez-Valle R, Green A, Ladogana A, Cuadrado-Corrales N, Mitrova E, Stoeck K, Sklaviadis T, Kulczycki J, Hess K, Krasnianski A, Equestre M, Slivarichova D, Saiz A, Calero M, Pocchiari M, Knight R, van Duijn CM, Zerr I. 2007. Influence of timing on CSF tests value for Creutzfeldt-Jakob disease diagnosis. J Neurol 254:901–906. https://doi.org/10.1007/s00415-006-0472-9.
46. Carswell C, Thompson A, Lukic A, Stevens J, Rudge P, Mead S, Collinge J, Hyare H. 2012. MRI findings are often missed in the diagnosis of Creutzfeldt-Jakob disease. BMC Neurol 12:153. https://doi.org/10.1186/1471-2377-12-153.
47. Eisenmenger L, Porter MC, Carswell CJ, Thompson A, Mead S, Rudge P, Collinge J, Brandner S, Jager HR, Hyare H. 2016. Evolution of diffusion-weighted magnetic resonance imaging signal abnormality in sporadic Creutzfeldt-Jakob disease, with histopathological correlation. JAMA Neurol 73:76–84. https://doi.org/10.1001/jamaneurol.2015.3159.
48. Wieser HG, Schindler K, Zumsteg D. 2006. EEG in Creutzfeldt-Jakob disease. Clin Neurophysiol 117:935–951. https://doi.org/10.1016/j.clinph.2005.12.007.
49. Shin JW, Yim B, Oh SH, Kim NK, Lee SK, Kim OJ. 2017. Redefining periodic patterns on electroencephalograms of patients with sporadic Creutzfeldt-Jakob disease. Clin Neurophysiol 128:756–762. https://doi.org/10.1016/j.clinph.2017.01.019.
50. Kovács GG, Puopolo M, Ladogana A, Pocchiari M, Budka H, van Duijn C, Collins SJ, Boyd A, Giulivi A, Coulthart M, Delasnerie-Laupretre N, Brandel JP, Zerr I, Kretzschmar HA, de Pedro-Cuesta J, Calero-Lara M, Glatzel M, Aguzzi A, Bishop M, Knight R, Belay G, Will R, Mitrova E, EUROCJD. 2005. Genetic prion disease: the EUROCJD experience. Hum Genet 118:166–174. https://doi.org/10.1007/s00439-005-0020-1.
51. Kovacs GG, Seguin J, Quadrio I, Hoftberger R, Kapas I, Streichenberger N, Biacabe AG, Meyronet D, Sciot R, Vandenberghe R, Majtenyi K, Laszlo L, Strobel T, Budka H, Perret-Liaudet A. 2011. Genetic Creutzfeldt-Jakob disease associated with the E200K mutation: characterization of a complex proteinopathy. Acta Neuropathol 121:39–57. https://doi.org/10.1007/s00401-010-0713-y.
52. Krasnianski A, Heinemann U, Ponto C, Kortt J, Kallenberg K, Varges D, Schulz-Schaeffer WJ, Kretzschmar HA, Zerr I. 2016. Clinical findings and diagnosis in genetic prion diseases in Germany. Eur J Epidemiol 31:187–196. https://doi.org/10.1007/s10654-015-0049-y.
53. Meiner Z, Gabizon R, Prusiner SB. 1997. Familial Creutzfeldt-Jakob disease. Codon 200 prion disease in Libyan Jews. Medicine (Baltimore) 76:227–237. https://doi.org/10.1097/00005792-199707000-00001.
54. Sano K, Satoh K, Atarashi R, Takashima H, Iwasaki Y, Yoshida M, Sanjo N, Murai H, Mizusawa H, Schmitz M, Zerr I, Kim YS, Nishida N. 2013. Early detection of abnormal prion protein in genetic human prion diseases now possible using real-time QUIC assay. PLoS One 8:e54915. https://doi.org/10.1371/journal.pone.0054915.
55. Spudich S, Mastrianni JA, Wrensch M, Gabizon R, Meiner Z, Kahana I, Rosenmann H, Kahana E, Prusiner SB. 1995. Complete penetrance of Creutzfeldt-Jakob disease in Libyan Jews carrying the E200K mutation in the prion protein gene. Mol Med 1:607–613. https://doi.org/10.1007/BF03401601.
56. Takada LT, Kim MO, Cleveland RW, Wong K, Forner SA, Gala II, Fong JC, Geschwind MD. 2017. Genetic prion disease: experience of a rapidly progressive dementia center in the United States and a review of the literature. Am J Med Genet B Neuropsychiatr Genet 174:36–69. https://doi.org/10.1002/ajmg.b.32505.
57. Amano Y, Kimura N, Hanaoka T, Aso Y, Hirano T, Murai H, Satoh K, Matsubara E. 2015. Creutzfeldt-Jakob disease with a prion protein gene codon 180 mutation presenting asymmetric cortical high-intensity on magnetic resonance imaging. Prion 9:29–33. https://doi.org/10.1080/19336896.2015.1017703.
58. Higuma M, Sanjo N, Satoh K, Shiga Y, Sakai K, Nozaki I, Hamaguchi T, Nakamura Y, Kitamoto T, Shirabe S, Murayama S, Yamada M, Tateishi J, Mizusawa H. 2013. Relationships between clinicopathological features and cerebrospinal fluid biomarkers in Japanese patients with genetic prion diseases. PLoS One 8:e60003. https://doi.org/10.1371/journal.pone.0060003.
59. Kong Q, Surewicz W, Petersen R, Zou W, Chen S, Gambetti P, Parchi P, Capellari S, Goldfarb L, Montagna P, Lugaresi E, Piccardo P, Ghetti B. 2004. Inherited prion diseases, p 673–776. *In* Prusiner SB (ed), Prion biology and diseases, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
60. Mutsukura K, Satoh K, Shirabe S, Tomita I, Fukutome T, Morikawa M, Iseki M, Sasaki K, Shiaga Y, Kitamoto T, Eguchi K. 2009. Familial Creutzfeldt-Jakob disease with a V180I mutation: comparative analysis with pathological findings and diffusion-weighted images. Dement Geriatr Cogn Disord 28:550–557. https://doi.org/10.1159/000254842.
61. Qina T, Sanjo N, Hizume M, Higuma M, Tomita M, Atarashi R, Satoh K, Nozaki

I, Hamaguchi T, Nakamura Y, Kobayashi A, Kitamoto T, Murayama S, Murai H, Yamada M, Mizusawa H. 2014. Clinical features of genetic Creutzfeldt-Jakob disease with V180I mutation in the prion protein gene. BMJ Open 4:e004968. https://doi.org/10.1136/bmjopen-2014-004968.
62. Breithaupt M, Romero C, Kallenberg K, Begue C, Sanchez-Juan P, Eigenbrod S, Kretzschmar H, Schelzke G, Meichtry E, Taratuto A, Zerr I. 2013. Magnetic resonance imaging in E200K and V210I mutations of the prion protein gene. Alzheimer Dis Assoc Disord 27:87–90. https://doi.org/10.1097/WAD.0b013e31824d578a.
63. Imbriani P, Marfia GA, Marciani MG, Poleggi A, Pocchiari M, Puoti G, Caltagirone C, Pisani A. 2016. Heidenhain variant in two patients with inherited V210I Creutzfeldt-Jakob disease. Int J Neurosci 126:381–383. https://doi.org/10.3109/00207454.2015.1047017.
64. Minikel EV, Vallabh SM, Lek M, Estrada K, Samocha KE, Sathirapongsasuti JF, McLean CY, Tung JY, Yu LP, Gambetti P, Blevins J, Zhang S, Cohen Y, Chen W, Yamada M, Hamaguchi T, Sanjo N, Mizusawa H, Nakamura Y, Kitamoto T, Collins SJ, Boyd A, Will RG, Knight R, Ponto C, Zerr I, Kraus TF, Eigenbrod S, Giese A, Calero M, de Pedro-Cuesta J, Haik S, Laplanche JL, Bouaziz-Amar E, Brandel JP, Capellari S, Parchi P, Poleggi A, Ladogana A, O'Donnell-Luria AH, Karczewski KJ, Marshall JL, Boehnke M, Laakso M, Mohlke KL, Kahler A, Chambert K, McCarroll S, Sullivan PF, Hultman CM, et al. 2016. Quantifying prion disease penetrance using large population control cohorts. Sci Transl Med 8:322ra9. https://doi.org/10.1126/scitranslmed.aad5169.
65. Nozaki I, Hamaguchi T, Sanjo N, Noguchi-Shinohara M, Sakai K, Nakamura Y, Sato T, Kitamoto T, Mizusawa H, Moriwaka F, Shiga Y, Kuroiwa Y, Nishizawa M, Kuzuhara S, Inuzuka T, Takeda M, Kuroda S, Abe K, Murai H, Murayama S, Tateishi J, Takumi I, Shirabe S, Harada M, Sadakane A, Yamada M. 2010. Prospective 10-year surveillance of human prion diseases in Japan. Brain 133:3043–3057. https://doi.org/10.1093/brain/awq216.
66. Shiga Y, Satoh K, Kitamoto T, Kanno S, Nakashima I, Sato S, Fujihara K, Takata H, Nobukuni K, Kuroda S, Takano H, Umeda Y, Konno H, Nagasato K, Satoh A, Matsuda Y, Hidaka M, Takahashi H, Sano Y, Kim K, Konishi T, Doh-Ura K, Sato T, Sasaki K, Nakamura Y, Yamada M, Mizusawa H, Itoyama Y. 2007. Two different clinical phenotypes of Creutzfeldt-Jakob disease with a M232R substitution. J Neurol 254:1509–1517. https://doi.org/10.1007/s00415-007-0540-9.
67. Zheng L, Longfei J, Jing Y, Xinqing Z, Haiqing S, Haiyan L, Fen W, Xiumin D, Jianping J. 2008. PRNP mutations in a series of apparently sporadic neurodegenerative dementias in China. Am J Med Genet B Neuropsychiatr Genet 147B:938–944. https://doi.org/10.1002/ajmg.b.30761.
68. Webb TE, Poulter M, Beck J, Uphill J, Adamson G, Campbell T, Linehan J, Powell C, Brandner S, Pal S, Siddique D, Wadsworth JD, Joiner S, Alner K, Petersen C, Hampson S, Rhymes C, Treacy C, Storey E, Geschwind MD, Nemeth AH, Wroe S, Collinge J, Mead S. 2008. Phenotypic heterogeneity and genetic modification of P102L inherited prion disease in an international series. Brain 131:2632–2646. https://doi.org/10.1093/brain/awn202.
69. Forloni G, Tettamanti M, Lucca U, Albanese Y, Quaglio E, Chiesa R, Erbetta A, Villani F, Redaelli V, Tagliavini F, Artuso V, Roiter I. 2015. Preventive study in subjects at risk of fatal familial insomnia: innovative approach to rare diseases. Prion 9:75–79. https://doi.org/10.1080/19336896.2015.1027857.
70. Krasnianski A, Bartl M, Sanchez Juan PJ, Heinemann U, Meissner B, Varges D, Schulze-Sturm U, Kretzschmar HA, Schulz-Schaeffer WJ, Zerr I. 2008. Fatal familial insomnia: clinical features and early identification. Ann Neurol 63:658–661. https://doi.org/10.1002/ana.21358.
71. Montagna P, Gambetti P, Cortelli P, Lugaresi E. 2003. Familial and sporadic fatal insomnia. Lancet Neurol 2:167–176. https://doi.org/10.1016/S1474-4422(03)00323-5.