

## THE 14-3-3 BRAIN PROTEIN IN CEREBROSPINAL FLUID AS A MARKER FOR TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHIES

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### ABSTRACT

**Background** There is no practical and reliable pre-mortem test for Creutzfeldt–Jakob disease and the related transmissible spongiform encephalopathies. Two proteins, designated 130 and 131, which have been detected in low concentrations in cerebrospinal fluid from patients with Creutzfeldt–Jakob disease, appear to be sensitive and specific markers for the disease. Attempts to identify these proteins, however, have been unsuccessful. We hypothesized that they may be present in the normal brain.

**Methods** We detected proteins 130 and 131 in normal human brain, partially sequenced their amino acids, and found that they matched the brain protein known as 14-3-3. We then developed a simple, rapid immunoassay for this protein and tested it in cerebrospinal fluid samples from 71 humans and 30 animals with spongiform encephalopathies and in control samples from 186 humans and 94 animals.

**Results** The immunoassay detected the 14-3-3 protein in cerebrospinal fluid from 68 of the 71 patients with Creutzfeldt–Jakob disease (96 percent; 95 percent confidence interval, 92 to 99 percent). Among 94 patients with other dementias, the specificity was 96 percent. If one excludes the three patients with dementia who had had strokes within one month before testing, the specificity was 99 percent. The test was positive in 12 of 24 patients with viral encephalitis. In animals the sensitivity of the assay was 87 percent and the specificity was 99 percent.

**Conclusions** In patients with dementia, a positive immunoassay for the 14-3-3 brain protein in cerebrospinal fluid strongly supports a diagnosis of Creutzfeldt–Jakob disease. This finding, however, does not support the use of the test in patients without clinically evident dementia. (N Engl J Med 1996;335:924-30.)

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**T**HE transmissible spongiform encephalopathies constitute a group of uniformly fatal neurodegenerative diseases. These diseases include Creutzfeldt–Jakob disease and kuru, among others, in humans,<sup>1,2</sup> scrapie in sheep and goats, and spongiform encephalopathy in cattle.<sup>3</sup> Spongiform encephalopathies are characterized by spongiform degeneration of the brain, reactive gliosis in the cortical and subcortical gray matter, and the presence of the abnormal isoform of the cellular prion protein.<sup>4</sup> These degenerative encephalopathies are transmissible when the infectious agent is experimentally inoculated into laboratory animals.

There is an urgent need for a pre-mortem diagnostic test that can identify humans and animals with transmissible spongiform encephalopathy. Such a test could be useful in patients with iatrogenically transmitted Creutzfeldt–Jakob disease<sup>3-5</sup> (especially because of difficulty decontaminating the infectious agent<sup>6</sup>), as well as in patients with the new strain of Creutzfeldt–Jakob disease possibly linked to bovine spongiform encephalopathy.<sup>7</sup> To date, a definitive diagnosis of transmissible spongiform encephalopathy requires histopathological examination of a brain-biopsy specimen. Brain biopsy, however, places patients and health care personnel at risk and may miss the site of disease.

Most cerebrospinal fluid proteins studied in patients with Creutzfeldt–Jakob disease have not proved useful diagnostically.<sup>8-11</sup> However, two 30-kd proteins detected by two-dimensional electrophoresis and designated proteins 130 and 131 correlate well with a diagnosis of Creutzfeldt–Jakob disease, with high sensitivity and specificity.<sup>12</sup> Identification of these proteins has clarified the pre-mortem diagnosis in several difficult cases,<sup>13-16</sup> but the assay technique is not practical for routine clinical use. The development of a simpler assay requires the identification and characterization of proteins 130 and 131, but initial attempts have been hampered by their low concentration in cerebrospinal fluid. We hypothesized that proteins 130 and 131 might be abundant in normal brain tissue, which would facilitate their identification and the development of an immunoassay that could be of use in establishing the diagnosis of Creutzfeldt–Jakob disease without the need for a brain biopsy.

### METHODS

#### Cerebrospinal Fluid Specimens

Cerebrospinal fluid specimens from humans or animals with possible diagnoses of transmissible spongiform encephalopathy or from well-studied controls with established diagnoses were submitted to the National Institutes of Health or to the California Institute of Technology. The diagnoses were made by the referring physicians or veterinarians according to standard clinical criteria, as well as pathological studies, as appropriate and available.

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The specimens from patients with possible Creutzfeldt–Jakob disease were assigned to one of three diagnostic categories on the basis of the available clinical or pathological information: pathologically confirmed, clinically definite (rapidly progressive dementia, myoclonus, and characteristic electroencephalographic findings), or clinically probable (progressive dementia; the presence of cerebrospinal fluid proteins 130 and 131; and myoclonus, ataxia, or characteristic electroencephalographic findings).<sup>17</sup> All samples were stored at  $-70^{\circ}\text{C}$  without preservative.

#### Purification and Characterization of Proteins 130 and 131

Sample preparation, two-dimensional electrophoresis, and image analysis of proteins from brain tissue were performed as described elsewhere.<sup>18–21</sup> The spot corresponding to protein 130 was excised from electroblotted membranes<sup>22</sup> and subjected to amino acid sequencing according to standard methods.<sup>23</sup> The partial amino acid sequences obtained were compared with those in the Swiss-Prot data bank with the use of the Basic Local Alignment Search Tool.

#### 14-3-3 Immunoassay in Cerebrospinal Fluid

All tests were conducted without knowledge of the diagnoses, and the method was adapted from that of Brown et al.<sup>24</sup> Fifty microliters of cerebrospinal fluid was mixed with 10  $\mu\text{l}$  of sample buffer (5 percent glycerol, 1 percent 2-mercaptoethanol, 1 percent sodium dodecyl sulfate, and a trace of bromophenol blue in the final solution) and boiled for five minutes. Samples were separated by sodium dodecyl sulfate–polyacrylamide-gel electrophoresis (4 percent stacking gel with 12 percent resolving gel at 75 V for three hours) and transferred to nitrocellulose. Immunostaining was performed by blocking with TRIS-buffered saline containing 0.3 percent Tween 20 for 30 minutes, followed by incubation with anti-14-3-3 $\beta$  polyclonal rabbit antibody (Santa Cruz Biotechnology, No. sc-629) at a 1:500 dilution and then by incubation with an alkaline phosphatase–conjugated antirabbit IgG antibody (BioSource International, No. ALI3405) at a 1:1000 dilution. Antigen was detected by colorimetric reaction. Molecular-weight markers and a positive control (cerebrospinal fluid from a patient with confirmed Creutzfeldt–Jakob disease) were included on every gel.

Statistical significance<sup>25</sup> was calculated with the chi-square test and Fisher's exact test for two-by-two contingency tables. P values of less than 0.05 were considered to indicate statistical significance. Confidence intervals were calculated with the exact method for a binomial parameter.<sup>25</sup>

## RESULTS

#### Identification, Characterization, and Verification of Proteins 130 and 131 as 14-3-3

The results of two-dimensional electrophoresis for the detection of cerebrospinal fluid proteins 130 and 131 in patients with Creutzfeldt–Jakob disease are shown in Figures 1A and 1B. Recent technical improvements have resulted in increased resolution, with six spots rather than the two (corresponding to proteins 130 and 131) originally described. In order to find an abundant source of these proteins, we examined normal brain tissue to determine whether any brain proteins are located near the constellation of spots corresponding to cerebrospinal fluid proteins 130 and 131. Figure 1C shows a region of a silver-stained gel after two-dimensional electrophoresis of normal brain proteins. Several of the proteins appear in the same area as the 130–131 constellation, including two that have the same charge and mass as the 130–131 constellation in cerebrospinal

fluid. The identification of the two spots on the basis of their position was confirmed by comigration studies. Spot 130 was purified by narrow-range two-dimensional electrophoresis (pH range, 4.5 to 5.4), as shown in Figure 1D.

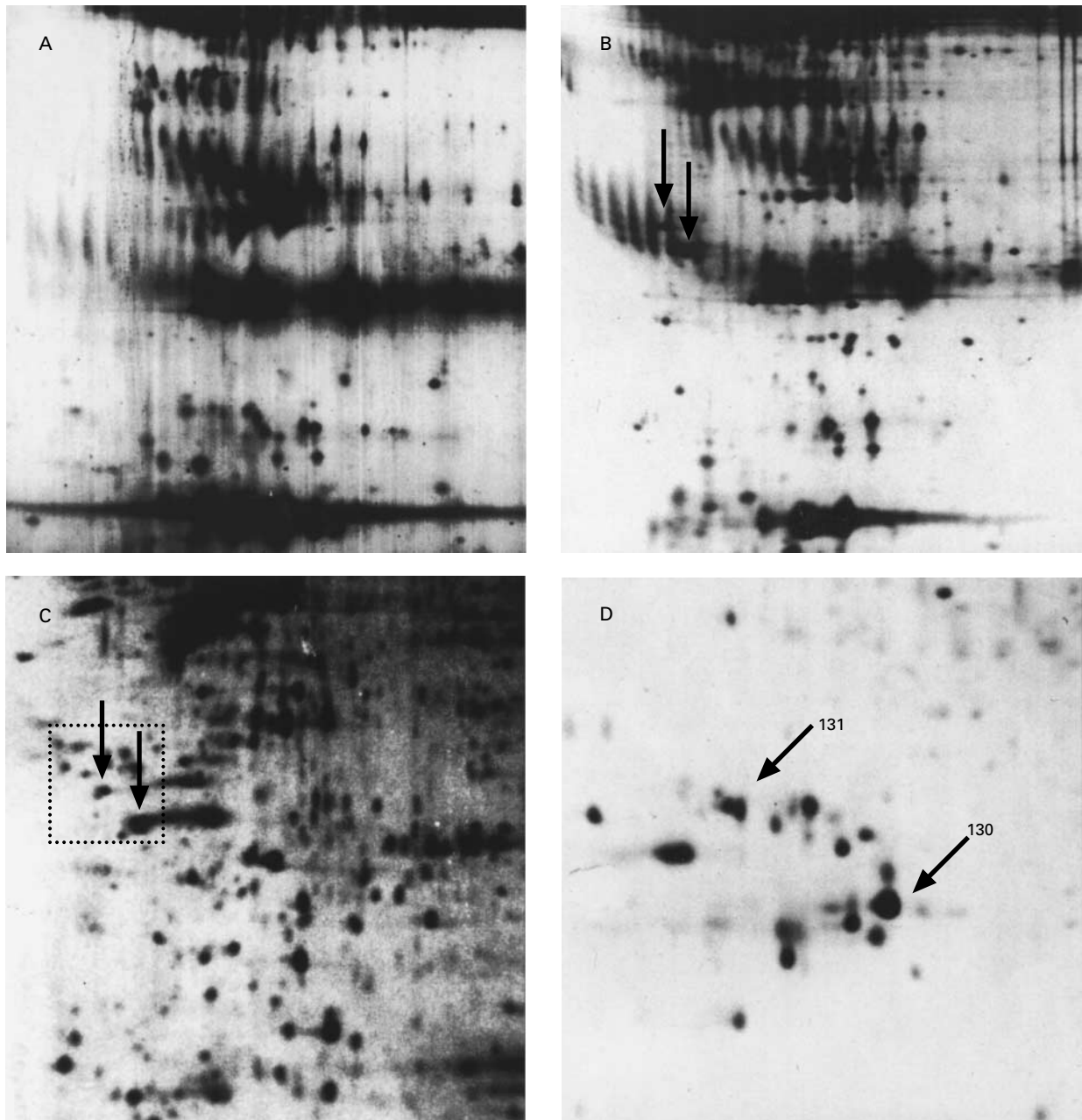
Spot 130 was excised from 10 blots, enzymatically digested, and microsequenced. Amino acid sequences were obtained from four peptide fragments. Three of these sequences, Val–Thr–Glu–Leu–Asn–Glu–Pro–Leu–Xaa–Asn–Glu–Asp–Xaa–Asn–Leu–Leu–Ser–Val–Ala, Asp–Tyr–Tyr–Xaa–Tyr–Leu–Ala–Glu–Val–Ala–Thr–Gly–Glu–Lys, and Asn–Val–Val–Xaa–Ala–Arg–Arg–Ser–Ser–Xaa–Arg–Val–Ile–Ser–Ser–Ile–Glu–Gln, matched the sequence of the human 14-3-3 protein, isoform  $\epsilon$ . The fourth sequence, Tyr–Ser–Glu–Ala–Xaa–Glu–Ile–Ser, matched the bovine 14-3-3 protein, isoform  $\gamma$ .

The 14-3-3 antibody reacted specifically with cerebrospinal fluid proteins 130 and 131 on a two-dimensional electrophoretic immunoblot but did not react with other cerebrospinal fluid proteins, thus verifying that cerebrospinal fluid proteins 130 and 131 are 14-3-3 proteins.

#### 14-3-3 Immunoassay

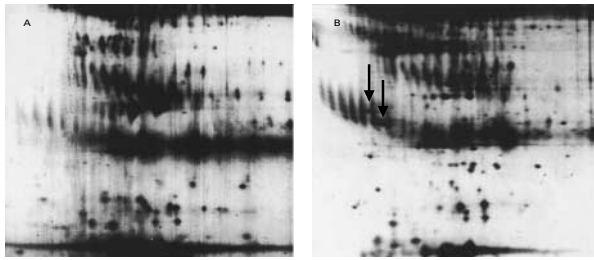
The discovery that the cerebrospinal fluid 30-kd spots (proteins 130 and 131) are 14-3-3 proteins led to the development of a simple immunoassay to aid in the diagnosis of transmissible spongiform encephalopathy. As Figure 2 shows, in cerebrospinal fluid from patients with Creutzfeldt–Jakob disease, there was a 30-kd immunoreactive band (lanes 2 and 3), whereas no such band was detected in cerebrospinal fluid from healthy controls (data not shown) or from a patient with Alzheimer's disease (lane 1). As expected, 14-3-3 was abundant in an extract of normal human brain (lane 8). The 14-3-3 protein was not found in normal serum (lane 6); however, it was also not detected in serum from patients with Creutzfeldt–Jakob disease (lane 7). Prion protein purified from the brain of a patient with Creutzfeldt–Jakob disease (lane 9) did not cross-react with 14-3-3 antibody, confirming that 14-3-3 is not prion protein.

Sixty-eight of 71 cerebrospinal fluid samples from patients with Creutzfeldt–Jakob disease (96 percent) were positive for 14-3-3 (Tables 1 and 2). Four of 94 samples (4 percent) from patients with other diseases involving dementia were positive for 14-3-3 (Tables 1 and 2) ( $P < 0.001$ ). Furthermore, when patients with dementia known to have had acute infarctions of the brain within one month before testing were excluded from the analysis, only 1 of 91 samples (1 percent) was positive ( $P < 0.001$ ). Samples from all 10 patients with multi-infarct dementia but without strokes in the month before testing were negative for 14-3-3. The single false positive result among the cerebrospinal fluid samples from the other patients with dementia was from a patient with



**Figure 1.** Regions of Silver-Stained Gels Showing Protein Spots 130 and 131 after Two-Dimensional Electrophoresis.

Panel A shows normal cerebrospinal fluid, with spots 130 and 131 absent. Panel B shows cerebrospinal fluid from a patient with Creutzfeldt–Jakob disease; the arrows point to spots 130 and 131. The gels in Panels C and D are from an extract of normal human brain. Panels A, B, and C show the same gel region, with isoelectric points ranging from 4.8 to 6.0 (left to right) on the x axis, and sizes ranging from 10 to 40 kd (bottom to top) on the y axis. The dotted box in Panel C outlines the region shown in Panel D from a gel with a pH range of 4.5 to 5.4. The arrows in Panels B and C indicate the location of spots 130 and 131, which are labeled in Panel D.



**Figure 2.** Immunostaining for Anti-14-3-3 $\beta$  Polyclonal Rabbit Antibody after Sodium Dodecyl Sulfate-Polyacrylamide-Gel Electrophoresis.

Lane 1 shows cerebrospinal fluid from a patient with pathological evidence of Alzheimer's disease, lanes 2 and 3 show cerebrospinal fluid from two patients with Creutzfeldt-Jakob disease, lane 4 shows cerebrospinal fluid from a normal cow, lane 5 shows cerebrospinal fluid from a cow with experimentally induced transmissible mink encephalopathy and pathological evidence of spongiform disease, lane 6 shows normal human serum, lane 7 shows serum from a patient with Creutzfeldt-Jakob disease, lane 8 shows an extract of normal human brain, and lane 9 shows purified prion protein from the brain of a patient with Creutzfeldt-Jakob disease.

a clinical diagnosis of Alzheimer's disease that had not been verified by pathological studies.

14-3-3 was detected in 18 of the 66 cerebrospinal fluid samples (27 percent) from patients with other neurologic illnesses not involving dementia. The 18 positive samples were from patients with acute viral encephalitis, stroke (without dementia) within one month before testing, subarachnoid hemorrhage, or Rett's syndrome. Creutzfeldt-Jakob disease could not reasonably be included in the differential diagnosis of any of these disorders.

Overall, the sensitivity of the 14-3-3 immunoassay as a marker for Creutzfeldt-Jakob disease was 96 percent (68 true positive results divided by 71 true positive and false negative results; 95 percent confidence interval, 92 to 99 percent), and the specificity was 88 percent (164 true negative results divided by 186 true negative and false positive results; 95 percent confidence interval, 84 to 92 percent). More important, the specificity of this assay among all the patients with dementia was 96 percent (90 true negative results divided by 94 true negative and false positive results; 95 percent confidence interval, 90 to 96 percent), and when the three patients with dementia and brain infarction within one month before testing were excluded, the specificity of the immunoassay was 99 percent (90 true negative results divided by 91 true negative and false positive results; 95 percent confidence interval, 97 to 100 percent) (Tables 1 and 2).

#### Comparison of Two-Dimensional Electrophoresis and Immunoassay

We compared the two-dimensional electrophoretic assay for proteins 130 and 131 with the 14-3-3

**TABLE 1.** CEREBROSPINAL FLUID SAMPLES EVALUATED WITH THE 14-3-3 IMMUNOASSAY, ACCORDING TO DIAGNOSIS.

DIAGNOSIS	TOTAL SAMPLES	POSITIVE SAMPLES
Creutzfeldt-Jakob disease	71	68
Pathologically confirmed	34	31
Clinically definite*	16	16
Clinically probable†	21	21
Other diseases involving dementia	94	4
Alzheimer's disease, pathologically confirmed	33	0
Alzheimer's disease, clinically diagnosed‡	16	1
Suspected Creutzfeldt-Jakob disease but with a negative 130-131 assay	12	0
Multi-infarct dementia without acute infarction§	10	0
Multi-infarct dementia with acute infarction§	3	3
Central nervous system lymphoma	1	0
Subdural hematoma	1	0
Niemann-Pick disease	1	0
Dementia associated with the acquired immunodeficiency syndrome	3	0
Huntington's chorea	3	0
Cortical basal ganglionic degeneration	1	0
Metabolic encephalopathy	1	0
Normal-pressure hydrocephalus	7	0
Parkinson's disease with dementia	2	0
Headache	7	0
Multiple sclerosis	8	0
Stroke without acute infarction§	4	0
Stroke with acute infarction§	4	4
Subarachnoid hemorrhage	1	1
Herpes simplex encephalitis	12	11
Other viral encephalitides	12	1
Neurosyphilis	2	0
Parkinson's disease	3	0
Psychiatric disorders	4	0
Rett's syndrome	6	1
Subacute sclerosing panencephalitis	3	0
Other disorders¶	22	0
Normal samples	4	0
Total	257	90

\*Clinically definite disease was defined as rapidly progressive dementia, myoclonus, and characteristic electroencephalographic findings.<sup>17</sup>

†Clinically probable disease was defined as progressive dementia; the presence of cerebrospinal fluid proteins 130 and 131; and myoclonus, a movement disorder, or characteristic electroencephalographic findings.<sup>17</sup>

‡A clinical diagnosis of Alzheimer's disease was based on the criteria of McKhann et al.<sup>26</sup>

§Acute infarction was defined as infarction within one month before testing.

¶Other disorders included amyotrophic lateral sclerosis, chronic inflammatory demyelinating polyradiculopathy, pseudotumor cerebri, Rasmussen's encephalitis, Schilder's disease, cerebral lupus erythematosus, Leigh's disease, myopathy, inborn errors of metabolism, Down's syndrome, moyamoya disease, congenital malformations, tuberous sclerosis, leukodystrophy, peripheral neuropathy, muscular dystrophy, and Wegener's granulomatosis.

**TABLE 2.** SENSITIVITY AND SPECIFICITY OF THE 14-3-3 IMMUNOASSAY FOR CREUTZFELDT-JAKOB DISEASE.

DIAGNOSIS	TOTAL SAMPLES	POSITIVE SAMPLES
	no. of samples (%)	
Creutzfeldt–Jakob disease	71	68 (96)
All other dementias	94	4 (4)
Other dementias, excluding those associated with infarction in the preceding month	91	1 (1)

Assay sensitivity, 96%; assay specificity, 99%\*

\*Among the patients who had dementia without infarction in the month preceding testing.

**TABLE 3.** COMPARISON OF TWO-DIMENSIONAL ELECTROPHORESIS FOR PROTEINS 130 AND 131 AND THE 14-3-3 IMMUNOASSAY.

DIAGNOSIS	TOTAL SAMPLES	POSITIVE SAMPLES	
		TWO-DIMENSIONAL ELECTROPHORESIS	14-3-3 IMMUNOASSAY
		no. of samples	
Creutzfeldt–Jakob disease			
Pathologically confirmed	5	5	5
Clinically definite*	10	8	10
Total	15	13	15
Other disorders			
Dementias	18	0	0
Other neurologic diseases	13	1	1
Total	31	1	1
Normal samples	4	0	0

\*Clinically definite disease was defined as rapidly progressive dementia, myoclonus, and characteristic electroencephalographic findings.<sup>17</sup>

immunoassay in 50 cerebrospinal fluid samples (Table 3). Thirteen of 15 specimens from patients with Creutzfeldt–Jakob disease were positive with both tests, and the other 2 samples were negative for proteins 130 and 131 but positive for the 14-3-3 protein. The results of the two tests were the same in samples from patients with dementias not associated with Creutzfeldt–Jakob disease or other neurologic disorders. Although the specificities of the two tests are similar, the 14-3-3 immunoassay has a slightly higher sensitivity.

#### 14-3-3 Immunoassay in Animals

The results of the studies in animals were consistent with those of the studies in humans. Figure 2

shows the assay results in cerebrospinal fluid from a normal cow (lane 4) and a cow with pathological evidence of transmissible spongiform encephalopathy (lane 5). As Table 4 shows, 14-3-3 protein was detected in cerebrospinal fluid from six of nine cattle with experimentally induced transmissible mink encephalopathy or scrapie. The one cow with clinical features but no pathological evidence of transmissible spongiform encephalopathy also had a positive test. No control cattle had positive assays. The test was positive in five of six sheep with naturally acquired scrapie and was negative in the one control sheep. All 15 experimentally infected chimpanzees had positive tests, whereas none of the 77 control chimpanzees did. The overall sensitivity of the 14-3-3 immunoassay in animals was 87 percent, and the overall specificity was 99 percent.

#### DISCUSSION

The discovery that proteins 130 and 131 belong to the 14-3-3 family of proteins has permitted the development of a premortem immunoassay of cerebrospinal fluid from humans and animals with transmissible spongiform encephalopathy. The overall specificity of the assay (88 percent) is low because we used a substantial number of cerebrospinal fluid samples from patients with various other conditions, including herpes simplex encephalitis, in which we expected 14-3-3 might be present in cerebrospinal fluid. When the assay was used more selectively, the specificity was very high (99 percent). This finding emphasizes the need to use the 14-3-3 marker as a test only in the appropriate clinical setting. For a patient with dementia, the detection of 14-3-3 in cerebrospinal fluid strongly supports a diagnosis of Creutzfeldt–Jakob disease, provided there has been no recognizable cerebral infarction within the preceding month. However, the one false positive result, in a patient with a clinical diagnosis of Alzheimer’s disease, suggests that some caution in interpretation is required until the assay has been evaluated in larger numbers of patients.

The predictive values of this test are governed not only by its sensitivity and specificity but also by the prevalence of Creutzfeldt–Jakob disease in the population tested. For example, in a population in which Creutzfeldt–Jakob disease has a prevalence of 1 percent, a positive test has a predictive value of only 49 percent, but in a population in which the prevalence of Creutzfeldt–Jakob disease is 50 percent, a positive test has a predictive value of 99 percent and a negative test has a predictive value of 95 percent. Therefore, this test will be most useful in patients with clinically suspected Creutzfeldt–Jakob disease. Thus far, the test cannot provide information on the clinical stage, severity, or source of the disease.

Although only a limited number of animals have been tested, the 14-3-3 immunoassay may prove to be a premortem diagnostic test for transmissible

**TABLE 4.** RESULTS OF THE 14-3-3 IMMUNOASSAY IN CEREBROSPINAL FLUID SAMPLES FROM ANIMALS.

ANIMALS	TOTAL SAMPLES	POSITIVE SAMPLES
	no. of samples	
Cattle		
With induced transmissible mink encephalopathy		
Positive pathological studies	5	3
Negative pathological studies	1*	1*
With induced scrapie	4	3
Normal controls	15	0
Sheep		
With naturally acquired scrapie	6	5
Normal control	1	0
Chimpanzees		
With induced transmissible spongiform encephalopathy	15	15
Normal controls	77	0
Total		
Animals with positive pathological studies	30	26
Animals with negative pathological studies	94	1
Overall assay sensitivity, 87%; overall assay specificity, 99%		

\*This cow had clinical symptoms of transmissible spongiform encephalopathy but normal histopathological studies. All other infected animals had positive histopathological studies.

spongiform encephalopathy in animals. At this stage, we have not studied animals with other neurologic disorders or cattle in the United Kingdom affected with bovine spongiform encephalopathy. However, the results in cattle with experimentally induced transmissible spongiform encephalopathy or naturally acquired scrapie in sheep suggest that this test will be of help in diagnosing bovine spongiform encephalopathy. At this time, the test cannot provide information on the clinical stage, severity, or source of the disease in animals.

We do not know why the detection of the 14-3-3 protein in cerebrospinal fluid is a useful and specific biochemical marker for transmissible spongiform encephalopathy. The role of 14-3-3 in the pathophysiology of Creutzfeldt-Jakob disease has yet to be determined. This highly conserved protein is found in a broad range of species, including yeast, plants, insects, and mammals, and has a wide variety of functions.<sup>27-36</sup> In humans and other mammals, 14-3-3 is a normal neuronal protein consisting of several isoforms, and it plays a part in the conformational stabilization of other proteins.<sup>29,33-36</sup> Since misfolded prion proteins are the central feature of Creutzfeldt-Jakob disease, an intriguing additional interpretation for the presence of 14-3-3 in cerebrospinal fluid from patients with this disease is that the protein may be centrally involved in the molecular pathologic features of transmissible spongiform encephalopathy.

We believe that the presence of 14-3-3 in cerebrospinal fluid may be due to massive neuronal disruption and the leakage of brain proteins into cerebrospinal fluid. Increased amounts of 14-3-3 in cerebrospinal fluid from some patients with inflammatory processes<sup>37</sup> and the high number of positive assays for 14-3-3 in patients with herpes simplex encephalitis (11 of 12) or recent infarctions (7 of 7) are consistent with this hypothesis. This possibility suggests that the quantity of 14-3-3 present in cerebrospinal fluid should be proportional to the rate and amount of neuronal destruction. We do not have supporting data in humans; however, studies with four experimentally inoculated chimpanzees indicate that 14-3-3 becomes detectable in the cerebrospinal fluid at or just before the onset of clinical signs of disease. Further experiments are required to determine the quantity of 14-3-3 detected in cerebrospinal fluid and the timing of its detection in relation to its clearance in transmissible spongiform encephalopathy.

In summary, we describe an immunoassay that may be of help in the premortem diagnosis of transmissible spongiform encephalopathy in humans and animals. This test can provide objective evidence for the diagnosis of Creutzfeldt-Jakob disease, particularly in the context of a rapidly progressive dementia accompanied by myoclonus or ataxia. The results in animals suggest that the 14-3-3 marker in cerebrospinal fluid reflects the pathological features of transmissible spongiform encephalopathy. The 14-3-3 immunoassay of cerebrospinal fluid can now be widely used to establish the diagnosis of transmissible spongiform encephalopathy in patients and animals.

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Drs. Hsich, Kenney, Gibbs, and Harrington have submitted a patent application based on this work. Dr. Harrington is the coinventor of the original two-dimensional electrophoretic test for transmissible spongiform encephalopathy (U.S. patent no. 4,892,814).

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## REFERENCES

- Brown P. Transmissible human spongiform encephalopathy (infectious cerebral amyloidosis): Creutzfeldt-Jakob disease, Gerstmann-Sträussler-Scheinker syndrome, and kuru. In: Calne DB, ed. Neurodegenerative diseases. Philadelphia: W.B. Saunders, 1994:839-76.
- Medori R, Tritschler H-J, LeBlanc A, et al. Fatal familial insomnia, a prion disease with a mutation at codon 178 of the prion protein gene. *N Engl J Med* 1992;326:444-9.
- Gajdusek DC. Infectious amyloids: subacute spongiform encephalopathies as transmissible cerebral amyloidosis. In: Fields BN, Knipe DM, Howley PM, eds. Fields virology. 3rd ed. Vol. 2. Philadelphia: Lippincott-Raven, 1996:2851-900.
- Prusiner SB. Prions. In: Fields BN, Knipe DM, Howley PM, eds. Fields virology. 3rd ed. Vol. 2. Philadelphia: Lippincott-Raven, 1996:2901-50.
- Brown P, Precece MA, Will RG. "Friendly fire" in medicine: hormones, homografts, and Creutzfeldt-Jakob disease. *Lancet* 1992;340:24-7.
- Asher DM, Gibbs CJ, Gajdusek DC. Slow viral infections: safe handling of the agents of subacute spongiform encephalopathies. In: Miller BM, ed. Laboratory safety: principles and practices. Washington, D.C.: American Society for Microbiology, 1986:59-71.
- Will RG, Ironside JW, Zeidler M, et al. A new variant of Creutzfeldt-Jakob disease in the UK. *Lancet* 1996;347:921-5.
- Jimi T, Wakayama Y, Shibuya S, et al. High levels of nervous system-specific proteins in cerebrospinal fluid in patients with early stage Creutzfeldt-Jakob disease. *Clin Chim Acta* 1992;211:37-46.
- Zerr I, Bodemer M, Räcker S, et al. Cerebrospinal fluid concentration of neuron-specific enolase in diagnosis of Creutzfeldt-Jakob disease. *Lancet* 1995;345:1609-10.
- Manaka H, Kato T, Kurita K, et al. Marked increase in cerebrospinal fluid ubiquitin in Creutzfeldt-Jakob disease. *Neurosci Lett* 1992;139:47-9.
- Awerbuch G, Peterson P, Sandky R. Elevated cerebrospinal fluid lactic acid levels in Creutzfeldt-Jakob disease. *Int J Neurosci* 1988;42:1-5.
- Harrington MG, Merrill CR, Asher DM, Gajdusek DC. Abnormal proteins in the cerebrospinal fluid of patients with Creutzfeldt-Jakob disease. *N Engl J Med* 1986;315:279-83.
- Croxson M, Brown P, Synck B, et al. A new case of Creutzfeldt-Jakob disease associated with human growth hormone therapy in New Zealand. *Neurology* 1988;38:1128-30.
- Blisard KS, Davis LE, Harrington MG, Lovell JK, Kornfeld M, Berger ML. Pre-mortem diagnosis of Creutzfeldt-Jakob disease by detection of abnormal cerebrospinal fluid proteins. *J Neurol Sci* 1990;99:75-81.
- Marzewski DJ, Towfighi J, Harrington MG, Merrill CR, Brown P. Creutzfeldt-Jakob disease following pituitary-derived human growth hormone therapy: a new American case. *Neurology* 1988;38:1131-3.
- Macario ME, Vaisman M, Buescu A, Neto VM, Araujo HM, Chagas C. Pituitary growth hormone and Creutzfeldt-Jakob disease. *BMJ* 1991;302:1149.
- Brown P, Cathala F, Castaigne P, Gajdusek DC. Creutzfeldt-Jakob disease: clinical analysis of a consecutive series of 230 neuropathologically verified cases. *Ann Neurol* 1986;20:597-602.
- Bjellqvist B, Sanchez JC, Pasquali C, et al. Micropreparative two-dimensional electrophoresis allowing the separation of samples containing milligram amounts of proteins. *Electrophoresis* 1993;14:1375-8.
- Bjellqvist B, Pasquali C, Ravier F, Sanchez JC, Hochstrasser D. A non-linear wide-range immobilized pH gradient for two-dimensional electrophoresis and its definition in a relevant pH scale. *Electrophoresis* 1993;14:1357-65.
- Harrington MG, Gudeman D, Zewert T, Yun M, Hood L. Analytical and micropreparative two-dimensional electrophoresis of proteins. *Methods Companion Methods Enzymol* 1991;3:98-108.
- Solomon JE, Harrington MG. A robust, high-sensitivity algorithm for automated detection of proteins in two-dimensional electrophoresis gels. *Comput Appl Biosci* 1993;9:133-9.
- Towbin H, Stachelin T, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc Natl Acad Sci U S A* 1979;76:4350-4.
- Aebersold RH, Pipes G, Hood LE, Kent SBH. N-terminal and internal sequence determination of microgram amounts of proteins separated by isoelectric focusing in immobilized pH gradients. *Electrophoresis* 1988;9:520-30.
- Brown P, Coker-Vann M, Pomeroy K, et al. Diagnosis of Creutzfeldt-Jakob disease by Western blot identification of marker protein in human brain tissue. *N Engl J Med* 1986;314:547-51.
- Rosner B. Fundamentals of biostatistics. 2nd ed. Boston: Duxbury Press, 1986.
- McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. *Neurology* 1984;34:939-44.
- Boston PF, Jackson P, Kynoch PAM, Thompson RJ. Purification, properties, and immunohistochemical localization of human brain 14-3-3 protein. *J Neurochem* 1982;38:1466-74.
- Aitken A, Collinge DB, van Heusden BPH, et al. 14-3-3 Proteins: a highly conserved, widespread family of eukaryotic proteins. *Trends Biochem Sci* 1992;17:498-501.
- Burbelo PD, Hall A. 14-3-3 Proteins: hot numbers in signal transduction. *Curr Biol* 1995;5:95-6.
- Robinson K, Jones D, Patel Y, et al. Mechanism of inhibition of protein kinase C by 14-3-3 isoforms: 14-3-3 isoforms do not have phospholipase A2 activity. *Biochem J* 1994;299:853-61.
- Ichimura T, Isobe T, Okuyama T, et al. Molecular cloning of cDNA coding for brain-specific 14-3-3 protein, a protein-kinase dependent activator of tyrosine and tryptophan hydroxylases. *Proc Natl Acad Sci U S A* 1988;85:7084-8.
- Morgan A, Burgoyne RD. Exo1 and Exo2 proteins stimulate calcium-dependent exocytosis in permeabilized adrenal chromaffin cells. *Nature* 1992;355:833-6.
- Freed E, Symons M, Macdonald SG, McCormick F, Ruggieri R. Binding of 14-3-3 proteins to the protein kinase Raf and effects on its activation. *Science* 1994;265:1713-6.
- Fantl WJ, Muslin AJ, Kikuchi A, et al. Activation of Raf-1 by 14-3-3 proteins. *Nature* 1994;371:612-4.
- Irie K, Gotoh Y, Yashar BM, Errede B, Nishida E, Matsumoto K. Stimulatory effects of yeast and mammalian 14-3-3 proteins on the Raf protein kinase. *Science* 1994;265:1716-9.
- Morrison D. 14-3-3: Modulators of signaling proteins? *Science* 1994;266:56-7.
- Boston PF, Jackson P, Thompson RJ. Human 14-3-3 protein: radioimmunoassay, tissue distribution, and cerebrospinal fluid levels in patients with neurological disorders. *J Neurochem* 1982;38:1475-82.

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