MINIREVIEW

A Critical Review of Atypical Cerebellum-Type Creutzfeldt-Jakob Disease: Its Relationship to "New Variant" CJD and Bovine Spongiform Encephalopathy

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Shortly after the appearance of bovine spongiform encephalopathy (BSE), Creutzfeldt-Jakob disease (CJD) was identified in young patients with nonclassical presentation such as difficulty in balancing and ataxia. The classical CJD in older patients starts with dementia. To distinguish between the two types, CJD in young persons has been termed "new variant" (nvCJD). The distinguishing features of classical CJD include initial presentation with dementia, confluent spongiform changes are very unusual in the cerebellum, and PrP plaques are rarely observed. For nvCJD, initially, difficulty with balancing and ataxia occurs, confluent spongiform changes are seen in the cerebellum, and a large number of PrP plaques are seen. The Icelandic observation of sheep scrapie revealed a predominantly ataxic form of scrapie, termed Type II, rather than the itchy form termed Type I. Both types have been known to exist in Europe. Since the clinical signs of Type II scrapie in sheep with trembling and ataxia are similar to those seen in BSE and nvCJD, this suggests that Type II is the cause of BSE and nvCJD. Over 8 years, from 1989 to 1996, I examined the clinical histories of 33 CJD cases aged between the ages of 18 and 84. Six under the age of 40 and 15 over the age of 40 had leading clinical features such as difficulty in balancing and ataxia similar to those seen in the young cases classified as "nvCJD." Brains were examined from the six of 15 cases over the age of 40, which revealed similar pathology to that seen in young patients classified as "nvCJD." These findings suggest that all age groups are susceptible to the strain of the agent derived from BSE cattle.

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All humans and animals, transmissible spongiform encephalopathies (TSEs) of the central nervous system (CNS) have common pathological features. Since bovine spongiform encephalopathy (BSE) emerged in 1985, similar TSEs have been diagnosed in domestic cats and captive wild animals in England (1, 2). TSEs have a common infectious agent with over 20 different strains (2–4). Common findings are vacuoles in the CNS, with each strain being identified by the vacuole distribution in the brain and by the incubation period. The BSE strain of the agent is unique in these properties.

A number of hypotheses on the nature of the agent have been proposed and discussed before (1, 2). Recently, the BSE Inquiry reported evidence regarding the nature of the TSE agent and the supposed role of mutation. Already it appears that the pronouncements of the BSE Inquiry have become conventional wisdom. Because of the overall importance of this matter, the nature of the agent and the evidence presented will be discussed in detail in a separate publication. In this paper, only the main points are contested, with supporting evidence in brief.

Protein Versus Virus Hypotheses

When attempting to trace the origins of an apparently new disease, the best first practice is to look for an agent that pre-existed in nature. Prusiner (5) considered that the individual prion, PrP^{sc}, is the agent. Professor Roger Morris in his evidence to the BSE Inquiry (*www.bse.org.uk*) suggested that a spontaneous mutation in a somatic cell of the prion protein gene in a single cow led to a sporadic case of BSE, while the feeding of this animal to other animals was the cause of the eventual epidemic. In addition, Dr. Jim

Hope suggested that mutation of the prion gene in somatic or germ cells of sheep resulted in the generation of a novel scrapie strain. It is important to stress that Dr. Hope is suggesting a mutation in the PrP gene of the germ cell of sheep, and another in the genome of the agent—not one, but two mutations. No evidence has been offered to support these theories, and it is, therefore, important to consider in brief the role of PrP and the mutation in the PrP.

Role of PrP

PrP 33 to 35 kDa, the precursor protein of PrP, is coded by a normal cellular house keeping (6). Evidence that PrP is not an essential component of the infectious agent has been discussed in detail in a previous review (1, 2, 4). Additionally, in a recent study involving transmission of the BSE agent to mice, it was found that although all of the mice injected with homogenate from BSE-infected cattle brain exhibited neurological symptoms and neuronal death, more than 55% of them had no detectable PrP^{sc} (7). Next, when brains of PrP^{sc-} mice were used to inoculate a second series of mice, most developed neurological signs, but a few presented the PrP^{sc-} pattern again. In the third pass, use of brains of PrP^{sc-} mice from the second pass transmitted the classic form, and almost all of the PrP^{sc-}-negative pattern except in one single mouse had disappeared. It is, therefore, clear that the BSE agent was replicating in the new host without benefit of PrP^{sc}. As the agent became adapted to the new host during serial passage, PrP^{sc} and vacuoles appeared, whereas in the first passage more than 55% of the mice had no detectable PrP^{sc}. Transmission of the BSE agent to humans will also be the first passage. Thus, if the data obtained in mice apply to the human, a large proportion of infected individuals might not have detectable PrP^{sc} or vacuoles and therefore would be misdiagnosed.

Role of Mutation in the PrP

Once it was realized that the PrP was host derived, a search began for mutations in the human PrP gene of affected families. Some of the familial Gerstmann-Sträussler-Scheinker syndrome (GSSS) cases have mutations in codon 129, and subsequently in codons 102, 117, 178, and 200. Some of these mutations have been seen in a number of first degree relatives of affected patients who are still healthy into their 60s and even mid-70s (8). But sporadic Creutzfeldt-Jakob disease (CJD) cases, which form the majority, do not have these mutations. Further, no mutation has been found in the PrP gene of cattle.

Recently, Balter (9) analyzed the protein-only hypothesis suggested by Prusiner; that is, familial CJD is linked to a spontaneous mutation in the PrP gene (10). Prusiner's group generated transgenic mice using multiple copies of mutant PrP gene referred to in humans as P102L. At the time many researchers thought that the protein-only hypothesis had been validated when two of the mutant mice expressing high levels of the PrP appeared to have spontaneously developed GSSS-like neurological symptoms. Later, however, it became apparent that the pathology was different, and the brain tissues had no infectivity (11).

Over the years, many lines of transgenic mice, with and without mutant in the PrP gene, have been generated and discussed in detail in the previous minireview (4). Neither transgenic mice in the presence of foreign PrP gene nor nontransgenic mice developed SEs or any other clinical neurological disorder. The most important finding was the comparison of transgenic and nontransgenic mice studies, which revealed that irrespective of PrP gene makeup with and without mutant, there is still a need for an infectious agent. These findings suggest that in GSSS, a spontaneous mutation in the PrP gene does not create PrP^{sc} or clinical disease.

Balter (9) also presented results of a study by Manson *et al.* from the meeting held in Tubingen, Germany, September 1999, where the authors repeated Prusiner's experiments with a new technique called double replacement gene. Unlike the Prusiner study, the transgenic mice expressing high PrP levels did not spontaneously develop the disease during their life span of about 900 days. However, when these mice were inoculated with infected-brain extracts, they did develop the clinical disease after an average of about 280 days.

The infectious agent starts replication only after inoculation of a new host species: monkeys or chimpanzees, pig, or mink. All these species have a different genetic makeup of the PrP (amino acid sequence) as compared with the donor host, which may be human, cow, or sheep. These affected monkeys/chimpanzees developed the clinical disease. Their PrP^{c} protein is modified into PrP^{sc} containing PrP not of man or cow (donor), but of monkey or chimpanzee (recipient-host). None of the point mutations from the donor host are copied in the PrP gene or in its product PrP^{sc} in the new host, but the clinical disease does develop, and the TSE agent replicates, maintaining its specific strain characteristic (4). Evidence from transmission studies has not revealed that the agent of slow virus spontaneously creates a *de novo* mutation in the PrP gene, nor do they copy any mutation in the PrP after being infected from one host to another.

Furthermore, it is evident from Prusiner's own transgenic mice studies that despite human PrP being expressed 4- to 8-fold higher in levels compared with normal human PrP^{c} levels, these transgenic mice with higher levels failed to develop PrP^{sc} containing human or human-mice hybrid PrP^{sc} (12). Prusiner's group, following these experiments in transgenic mice, have concluded that another "X" protein, that is, a macromolecular chaperone other than PrP^{sc}, is required in the post-translational process (12), as previously suggested by Narang (13). The final conclusion of these findings suggests that PrP itself is not the agent, therefore, the infectious agent is something other than PrP^{sc}. Indeed, Prusiner's group, from their studies, concluded that the host-encoded, nonpathogenic precursor PrP^{c} is modified into PrP^{sc} with the help of the "X" protein in a new host.

This, along with other evidence I have presented, demonstrates that blanket acceptance of the hypotheses proposed by the BSE Inquiry is dangerous both for science and for the proper evidence-based investigation of the agent.

A search of the literature shows two distinct clinical syndromes in sheep, both of which have been called scrapie (1, 2). Type I is manifested by itchiness and loss of wool (the common type). Type II is manifested by trembling and ataxia. When sheep are inoculated with brain tissue from cows with BSE, they develop trembling and ataxia (Type II scrapie). The major clinical signs (trembling and ataxia) in BSE, kuru, and many of the recent cases of CJD are identical. Evidence suggests that Type II is the cause of BSE, CJD, and of kuru. The BSE strain has shorter incubation periods and appears very efficient in infecting other species by the oral and intracerebral routes. A single infected BSE cow, whether expressing symptoms or not, on entering the human food chain could potentially infect a large number of humans.

CJD

For CJD, the incidence peaks between the ages of 55 and 75 years (14). However, some exceptions have been reported among more than 3,000 cases world-wide, and nine autopsy-verified cases have been documented in patients under 30 years old, the youngest being 16. Three of these "under-aged" CJD patients were well-known cases of surgical transmission (15, 16). The incidence of sporadic CJD in people under the age of 40 has been established to be about 1 per 20 million. In the U.S., in ages under 30, the first three cases of CJD were linked to contaminated growth hormone, which occurred within 1 year in 1985. From the world-wide statistical figures of sporadic cases, the chance of three CJD cases occurring in 1 year in this age group would be approximately 1 in 10^{12} (1). Since this time, the appearance of BSE and CJD has been confirmed in a large number of "under-aged" patients in England.

History of CJD

CJD was first recognized as a specific disease in 1921 (17). Typically, CJD presents itself as a progressive mental deterioration similar to those seen in cases of Alzheimer's disease (AD) and, therefore, the two diseases cannot always be distinguished while the patient is alive. AD is a nontransmissible dementing disorder; however, evidence for and against the transmissibility of AD and amyotrophic lateral sclerosis has been presented (18–21). Smith *et al.* (22), in a retrospective neuropathological study of brains from 66 patients with AD, demonstrated vacuolar changes in 50 cases (76%), which they described as almost indistinguishable histopathologically from spongiform changes characteristic of CJD.

As is plain from published studies, the term CJD used by many authors has covered a wide spectrum of clinical symptomatology and neuropathology features that are known to vary from case to case. Heidenhain (23) reported the disease in a paper entitled "Concerning a Peculiar Organic Disease of the Presenium." The first paper entitled "A Rapidly Progressive Presenile Dementia of the Jakob Type" was published by Jervis (24). Later, Meyer *et al.* (25), under the title "A Rare Presenile Dementia Associated with Cortical Blindness" (Heidenhain's Syndrome), described another example of this disease. Meyer asserted that Heidenhain regarded the condition as closely related to the "Creutzfeldt-Jakob syndrome" and also belonging to the "Creutzfeldt-Jakob" syndrome. In order to distinguish it from other varieties of this disease, they suggested that the name "Heidenhain's Syndrome" be used to designate their case and all similar cases.

There are three groups of this disease: familial, iatrogenic CJD, and sporadic. In the familial group, these cases occur within the same family and represent approximately 5% to 15% of CJD (26). The major distinguishing features of familial disease are the earlier age at onset and the longer duration of illness. In general, the patients with familial CJD do not differ appreciably in their clinical symptomatology, neurological, and neuropathological features from sporadic CJD. Some familial cases are classified as GSSS. This rare variant of CJD was first described in an Austrian family in 1936. Subsequently, GSSS has been reported in about a dozen other unrelated families as well as in sporadic form. GSSS, which is pathologically similar to CJD, in almost all cases appeared to show a pattern that is consistent with the autosomal dominant inheritance pattern (27). In GSSS patients, the first clinical sign of illness is likely to be an episode of confusion, vertigo, diplopia, or blurred vision followed by tremor, paresis, or paresthesia in one of the limbs. These signs precede dementia and progress as an atypical dementia with generalized tremors (28, 29). Many of the symptoms clinically and pathologically overlap with those of kuru and CJD. EEG in these cases is within normal limits. Histopathologically, a large number of unusual forms of amyloid plaques are seen throughout the brain. GSSS cases have been demonstrated to be infectious by experimental transmission of disease to nonhuman primates with extracts of brain tissue from human patients, suggesting that the agent may be carried via a germline, as in AIDS, and is not a genetic disease. Vertical transmission also has been observed in BSE cattle. Recently, CJD has been reported in a mother and baby daughter in England, suggesting vertical transmission in humans.

The iatrogenic CJD group constitutes a small percentage and this type is caused by accidental inoculation (through contaminated instruments) and through the use of contaminated human growth hormone (hGH). Since 1985, more than 65 cases of CJD have been described among recipients of pituitary-derived hGH. These cases have been reported in the United States, England, and France. The clinical presentation in the hGH recipients of CJD cases starts with cerebellar function deterioration with severe ataxia, especially of the legs, while mental deterioration is a late manifestation. These symptoms seen in hGH recipients

were also typical of kuru cases. The EEG was consistent with a diffuse encephalopathy, although periodic discharges were absent. It is interesting to note that the clinical presentation of these hGH patients also resembles that of the "new strain" of CJD cases described in this paper.

The sporadic CJD group, which forms the majority of cases, is rare, but is found world-wide. The annual incidence of classic CJD has been estimated to be one per one million of the human population. However, published data shows that the prevalence of CJD varies markedly from one country to another. The annual incidence among ethnic groups in some countries may reach more than one per one million, while in the general population in the U.S., it ranges from 0.26 to 0.4 (30), in France, 0.32 (31), and in England 0.09 (28). Since BSE appeared, the incidence of all CJD cases in England has increased year by year from 33 in 1990, to 36 in 1991, and to 88 in 1998. It is obvious from personal correspondence with the Medical Research Council that not all cases of CJD are referred to the Surveillance Unit and, as the disease is not required to be reported in England, the incidence of CJD may be underestimated. However, the available data indicate that the incidence of CJD in young people increased from three in 1995 has gone up to 18 in 1998. Andrews *et al.* (32) reported 14 deaths in the first half of the year 2000 compared with 13 for the all of 1999—an increase of over 100%. At the same time, another six patients are ill, and it is possible that additional cases from 1999–2000 are yet to be identified. The overall incidence might appear to have increased due to improved ascertainment surrounding extensive publicity. However, since BSE came into the public domain in 1988 and the Surveillance Unit was set up in 1990, I believe this explanation is unlikely.

CJD presents a broad variety of clinical manifestations with a range of clinical syndromes involving dementia during middle and late life. Compared with Alzheimer's disease, CJD follows a more rapid course over a period of 4 to 7 months and is almost invariably accompanied by a variety of neurological abnormalities, especially visual, cerebellar, and extrapyramidal deficits, often in association with myoclonus and other involuntary movements.

Brown *et al.* (29) summarized a series of 300 CJD cases, all confirmed by experimental transmission. In the great majority of patients, the onset of the illness has been reported to be rather gradual. Brown *et al.* (29) measured this period in weeks to months, but found that some of the patients experienced a rapidly progressive, or even sudden, onset. The first sign of the illness was often an episode of confusion, vertigo, diplopia, or blurred vision. Patients with the more typical subacute onset were reported to have a gradual failure of memory taking the form of an inability to remember names or recent events, losing one's way in familiar surroundings, or general confusion. Behavioral abnormalities were usually a consequence of an agitated or depressed state that began during the prodromal period.

The important diagnostic symptoms of CJD are myoclonus, mental deterioration, and EEG pattern of slow tracing. In about 50% of CJD cases, EEG-specific pattern changes are important diagnostic indicators. However, in 25% of sporadic, and in almost all the new variant of CJD cases, the EEG pattern changes appear only in the advanced stage of the illness when patients are no longer able to stand or walk.

Odd Cases of Sporadic CJD

Foley *et al.* (33) reported three cases of subacute progressive encephalopathy occurring in middle aged patients under the heading "The Ataxic-Cerebellar Form of CJD." Later, Brownell *et al.* (34) described four additional cases of the ataxic-cerebellar form of CJD and reviewed six previous cases. The outstanding clinical features, in order of their appearance reported, were rapidly progressive ataxia of cerebellar with imperfect articulation of speech due to disturbances of muscular control (dysarthria), which resulted from damage to the central or peripheral nervous system; involuntary rhythmic jerking movements, dementia progressing to coma and, finally, a state of generalized muscular rigidity in which the involuntary movements tended to disappear. In the final stage, speech was totally lost.

Subsequently, several more cases with initially ataxic symptoms of CJD have been reported in Europe, the U.S., and Japan. In this ataxic-cerebellar form of CJD, the symptoms appear to start in the same way as those observed in kuru patients. As the ataxia becomes progressively more severe, a distinctive tremor becomes apparent. Alema *et al.* (35), Khochneviss (36), and Siedler *et al.* (37) noted 60 to 70 cases of the ataxic-cerebellar form (which had appeared in the literature), but there is no agreement as to which of these cases should be included in the CJD group. Khochneviss (36) placed these in a separate category of "subacute SE," a term introduced by Jacob *et al.* (38), leaving open the question: Is the vascular pathology in such cases, or the secondary effects of the patient's terminal state, or just mere coincidence the principal cause of the cerebral changes?

Since status spongiosus has been repeatedly observed in these mixed cases without vascular changes usually being demonstrable, vascular changes cannot be the primary cause.

In many CJD cases, death occurs within 3 to 6 months, but cases have been recorded with deaths resulting after a 10- and 16-year clinical duration of the illness (39). The main distinguishing factor that differentiates CJD from AD and other neurological disorders, as in other SEs, is the existence of a transmissible agent in all CJD cases. Since the appearance of BSE, Narang (40) has reported four "atypical" cases with unusual clinical course with leading clinical features such as difficulty in balancing and ataxia in 47- to 84-year-old patients who have histopathological lesions similar to those observed in BSE cattle brain, but different from those seen in typical sporadic CJD cases (40, 41). Some of the early symptoms include behavioral and mood changes, along with depression. Balancing and walking be-

come difficult and the patients feel as if they are going to fall and need support. These symptoms become more marked with a swaying and weaving gait—the patient tending to trip and stumble—and are similar to those seen in the ataxic-cerebellar form of CJD and in kuru. Like kuru, none of these cases had the typical EEG patterns traditionally associated with CJD. During the last stage, EEG results showed some slow amplitude activity. Furthermore, because symptoms were so different from typical CJD, these patients were referred to a psychiatrist.

In England during 1994 and 1995, 10 cases of CJD were reported in relatively young persons where the clinical course was not typical of sporadic CJD (42). Their age, clinical manifestations, and unique neuropathology distinguished them from "classical CJD," and the term new variant CJD (nvCJD) was introduced in England.

It is a well-established fact that the strains of the TSE agent "breed true" with their own particular biological properties such as incubation period and distribution of lesions (1, 2). Since 1961, based on clinical observations, two strains of scrapie have been readily recognizable in sheep: Type I, "itchy" and Type II, "trotting" type (1, 2, 43, 44). The Icelandic observation of sheep scrapie, which revealed a predominantly ataxic and has been termed Type II, saw clinical symptoms that are quite distinct from the itchy form termed Type I. Now, Dr. Stanley Prusiner has also suggested that "the agent has always been in sheep and probably always will be. Lurking out there in small numbers in sheep may be the BSE prion, and under the right circumstances, the BSE prion emerges" (45). Throughout the world, Type I "itchy" scrapie, with wool loss, is the most common. Calves inoculated with Type I strain develop mild spongiform changes in their brain, but the clinical signs are not similar to those observed in BSE. Sheep experimentally inoculated or fed with brain tissues from cows with BSE develop Type II scrapie, exhibiting clinical signs very similar to BSE, in particular, locomotor incoordination, trembling, lethargy, and ataxia.

Like scrapie, classic CJD cases can be divided into two groups based on clinical symptoms. Group I includes the classic sporadic CJD cases and is caused by accidental inoculation with Type I scrapie. Not all humans will develop CJD in their life span.

Group 2 CJD has been termed "new variant" (nvCJD) and is very likely to have been the consequence of eating the BSE strain of the agent. Sheep inoculated with brain tissues from cows with BSE exhibit clinical signs very similar to the Type II scrapie sheep with trembling and ataxia. These signs are similar in BSE, nvCJD, kuru, and in cases of the ataxic-cerebellar form of CJD described by Foley *et al.* (23) and Brownell *et al.* (24) 20 to 30 years before BSE appeared. In summer 2000 at Birmingham, Dr. S. Prusiner stated that his colleague, Dr. Mike Scott, believes that sheep carry two strains: the scrapie strain and the BSE strain of the agent (45). This would suggests that Type II is the cause of BSE and nvCJD. Therefore, the name nvCJD is misleading.

The Link between BSE and CJD

In its 1999 annual report, the National CJD Surveillance Unit in Edinburgh failed to find a positive link between nvCJD and eating beef. CJD, like other spongiform diseases, has a very long symptom-free incubation period of years, making it infeasible to compare dietary histories of known victims with those patients who were not suffering from disease, as the Surveillance Unit did, as if they were investigating some ordinary form of food poisoning. Can you remember what you or a relative ate last week, let alone 10 years ago? The Surveillance Unit seems also to have forgotten that beef is not all that is infected; the BSE agent has infected many other farm animals.

To the contrary, I contend that there is good evidence that nvCJD is a consequence of consuming BSE-contaminated foodstuffs. Virus strains are identified in the laboratory experiments, some by injecting animals. In the past few years, different groups of laboratory mice have been injected with brain tissue from BSE cows, from cats with feline spongiform disease, from various zoo animals since the BSE outbreak, and from a few humans who died of nvCJD. In each case, the disease showed identical properties: the same incubation period and the same distinctive pattern of vacuoles in the brain, all demonstrating a common origin of the agent (1, 4).

The pathological difference between "classical CJD" and nvCJD is diagnosed by histopathological microscopical examination of the brain sections. In nvCJD brains, the pathological changes are distinctive, with spongiform changes accompanied by neuronal loss and reactive gliosis. A particularly striking feature is the damaged cerebral cortex, especially in the occipital lobes. Variation in the intensity, the distribution of lesions, and the stage of development from case to case are evident from the reported cases. However, confluent spongiform changes are very unusual in the cerebellum in sporadic CJD cases, which was one of the outstanding distinguishing features of nvCJD. Furthermore, the presence of PrP-positive plaques is another distinguishing feature of patients who were supposed to have become infected with the BSE strain of the agent (1, 28, 42).

Plaques

Based on immunohistochemical staining process, two types of plaques have been identified in CJD brain tissue. Amyloid β-protein positive (APP) plaques, termed amyloid plaques, are a "hallmark" of AD and Down's syndrome. When the amyloid precursor protein is sliced into three segments, the middle segment amyloid β-protein, a 4-kDa protein, forms plaques, which are seen in brain sections, while the other two segments of the precursor protein have not been demonstrated. A small number of amyloid plaques have been observed in about 15% of CJD cases. An ion-capture technique was used to demonstrate amyloid precursor protein, or its segments, in clinically diagnosed cases of AD. This involves concentration of APP from urine

samples, and the concentrate was subjected to Western blotting. Using APP antibody 369, it was possible to demonstrate one, two, or all three APP segments from urine of AD cases, but not from healthy individuals (Fig. 1), and this may provide a significant contribution for diagnostic work in relation to CJD and nvCJD. Similarly, this technique could be applied using PrP antibody to demonstrate protease-resistant protein/SAF in BSE and CJD by Western blotting, or a concentrated sample can be used to prepare grids for electron microscopic examination (Fig. 2).

Protease-resistant protein- (PrP 27–30 kDa) positive plaques derived from PrP33–35 kDa precursor protein (PrP^c) coded by a normal gene are seen in about 10% of sporadic CJD cases, 50% to 70% kuru patients, and in all GSSS patients (46). It is important to point out that PrP-positive plaques have not been observed in AD or Down's syndrome or any other non-SE neurological disorder.

A review of the clinical history of 33 CJD cases with patients aged between 18 and 84, backed by histopathological evidence, has revealed that older patients have died from 1989 to 1996 from infection by the BSE strain of the agent. Humans of all age groups would therefore seem to be susceptible to this strain of the agent. Evidence presented also demonstrates that a small percentage of CJD cases are being caused by accidental use of contaminated blood from CJD victims. Clinical histories of these cases have been described in detail (28).

Selected Case Historie

B.D., an ambulance driver, died at the age of 41 after an illness that lasted 11 months. His symptoms started with depression, followed by muscular pains in his arms and legs. His family doctor attributed his symptoms to arthritis. The first thing his wife noticed was that he was frightened and refused to get into a bath. At home he would say, "My balance is all off." He could not touch his nose and was having difficulty in walking and balancing. Whenever he stood up, he seemed to wobble. He could not walk without support. He described his condition as similar to being drunk on alcohol. He started to hallucinate and lost his speech. His shakes were similar to those seen in BSE cattle, and he had diagnosed himself as suffering from "mad cow disease." His whole personality changed, but he never became aggressive. He slept more and more. His mental confusion didn't come until later. Although his symptoms and brain pathology were typical of being infected with the BSE strain of the agent, his wife was told by his doctors that he could not have BSE because of his advanced age.

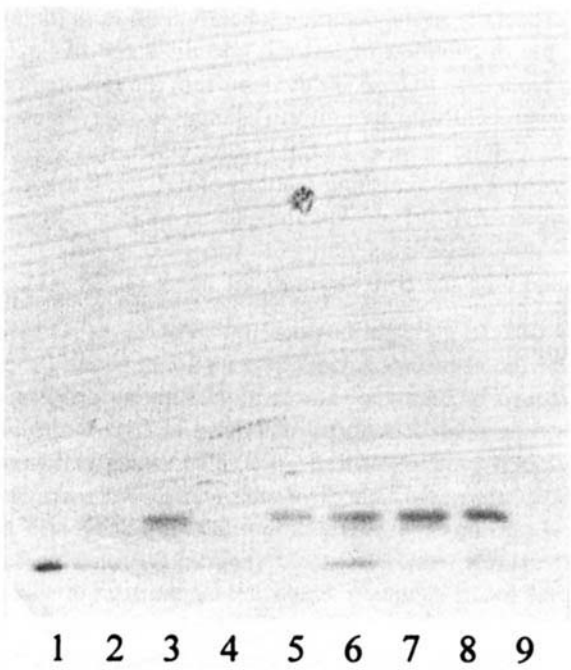

Figure 1. β-Amyloid protein (APP) was concentrated from urine specimens of AD patients and Western blot was performed. The blot was stained by APP-antibody 369. Positive results are seen in lane 1, control APP; lanes 2, 3, 5, 6, 7, 8, specimens from AD patient; lane 4, Parkinson's disease; and lane 9, healthy control.

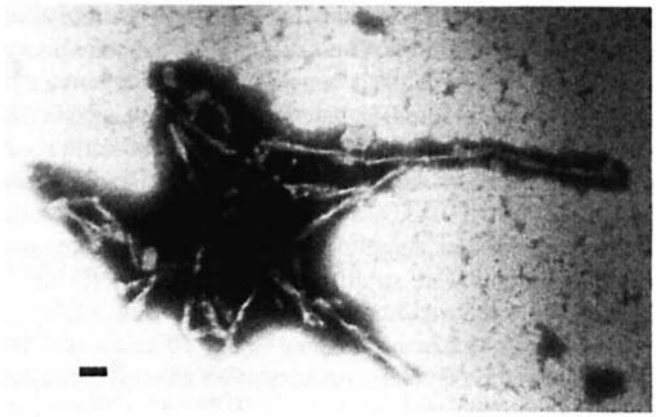

Figure 2. Nemavirus/SAF were concentrated from urine specimens of a 19-year-old CJD patient. Grids were prepared by a low speed centrifugation, washed with 1% solution of SDS, and negatively stained and examined by electron microscope. Note typical SAF. Grid bar = 100 nm.

J.D. and E.B.

Two sisters, aged 51 and 59, both developed the clinical symptoms about 6 years apart. They lived together for 42 years, but were separated for the last 10 years. The family related their clinical symptoms to BSE cows on their farm. J.D. died first in 1989. She woke up one morning with her shoulder hurting. Her family doctor diagnosed a trapped nerve in her shoulder and suggested that she might have to wear a collar. Within days, she developed a weakness in her legs and found it difficult to walk and was treated with antidepressants. Her neurologist initially suggested Huntington's Chorea. In the hospital, after an EEG test, CJD was confirmed. The family had heard of Mad Cow disease because it had occurred on their farm, but they had not heard of CJD. J.D. had been a blood donor since 1981, about 9 years. No post-mortem was carried out.

In September 1996, the second sister, E.B., after an illness that lasted over 18 months, also died at the age of 59 suffering with CJD. She developed a slight tremor in her hands that appeared similar to early symptoms of Parkin-

son's disease, which gradually progressed to staggering and loss of balance. She reminded the family once again of BSE in their cattle, and of people with polio. Clinical symptoms were typical of the BSE strain of the agent. Unlike her sister who was a blood donor, E.B. was a recipient of blood and had received fairly large volumes of blood over a 4-year period. The author tried to trace E.B.'s blood donors. Out of five, only three were traceable, and these were fit and healthy. A comparative histopathological examination of the brain revealed extensive vacuolation of the cerebellum and numerous PrP-positive plaques. The PrP plaques were much smaller in size compared with the patients infected with the BSE strain of the agent, but were similar in size and distribution in the cerebellum to those observed in human growth hormone cases (Fig. 5). In neither instance, of course, is there a species barrier, and the small size of plaques in both instances suggest a fast onset of disease. This identical clustering distribution of PrP plaques in the brain of patient E.B. strongly suggests that this case was infected through blood transfusion.

K.H.

A diesel fitter became ill at the age of 41. According to his wife, he was a sociable, fun-loving person. His illness progressed over a period of 18 months. He became depressed and began to lose his coordination. He developed a tremor and became very clumsy with food. He became very restless and very fidgety and couldn't keep still, always crossing and uncrossing his legs and moving his position in the chair. He used to fall asleep a lot when he came home from work. He would get annoyed with himself, but just didn't know why.

The family doctor diagnosed a clinical depression and K.H. was put on antidepressants. He used to take any news of disasters or anything sad on television as if it were a personal disaster to himself. When he kept telling his family doctor how frightened he was that he was going mad, the doctor tried to reassure him that he was not. His perception was changing. When walking down the corridor, he would duck under the doorframe as if in some alarm or as if the space was too small for him to go through. Similar behavior, nervousness and spatial confusion, has been observed in cattle with BSE.

He developed a stagger and had difficulty in walking that necessitated him using a walking stick. This would also let other people know that he was not drunk. At this stage, he was still driving, although his driving was really quite jerky, and he sometimes had difficulty changing gears. He actually took a friend out for a drive and she noticed and commented on his erratic driving. That worried him. His outings became more and more rare as the disease got the upper hand. His appetite seemed to fall off, and he lost about a stone in weight. At about the same time, his sex drive decreased. He said he "didn't have any thoughts or anything in his head." He just felt as if he wanted to cry.

The first EEG result was within normal limits, while a repeat EEG showed a considerable change. His speech was becoming hesitant and quite jerky. He was having terrible shakes and jerks. His wife tried to cuddle him in order to soothe him, and tried to stroke his back and do the things that would normally calm him down. He seemed to enjoy listening to music and he would make noises to it as if he was trying to sing to it. Eventually, he would fall asleep. He had been a regular blood donor and the final occasion on which he had donated blood was only some 6 months before the appearance of his symptoms. According to his record book, he might have given blood while he was ill. He had an appointment to do so during this period, but his wife was not sure whether or not he had kept it.

Histopathological Studies

Blocks from the frontal, temporal, parietal, and occipital cortex, basal ganglia, thalamus, hypothalamus, and cerebellum were fixed in formalin. Before routine processing into paraffin wax, tissues were immersed in 96% formic acid for 1 hr. Sections were cut at 55 m and were stained by conventional histopathological techniques. For immunocytochemistry, sections were treated with 96% formic acid for 5 min and were then incubated with PrP antibodies (KG9 and 3F4) (42).

The results of molecular genetic analysis of these cases showed a mutation in the PrP precursor protein gene. In all six cases, leading clinical features included difficulty in balancing and ataxia and extensive vacuolar lesions were seen in the cerebral cortex and cerebellum (Fig. 3). In addition, the most consistent striking neuropathological feature was the staining of PrP plaques varying in size from 2 to 35m (Fig. 4, A and B). Immunohistochemical staining showed that pericellular PrP plaques were extensively distributed throughout the cerebrum and cerebellum, with smaller numbers in the basal ganglia, thalamus, and hippocampus. Often, plaques were seen in the absence of confluent spongiform changes in the surrounding neuropil. The pattern distribution of PrP plaques in the cerebral cortex and

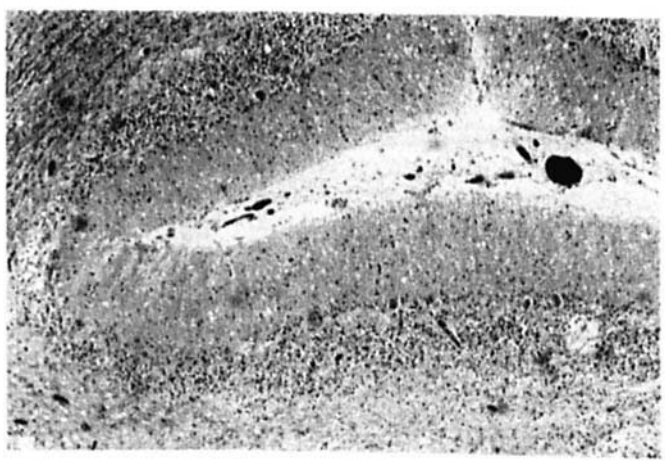

Figure 3. Spongiform changes in the cerebellum from 53-year-old case showing multiple small vacuoles in the molecular layer showing extensive vacuolation. Haematoxylin and eosin stain, ×126.

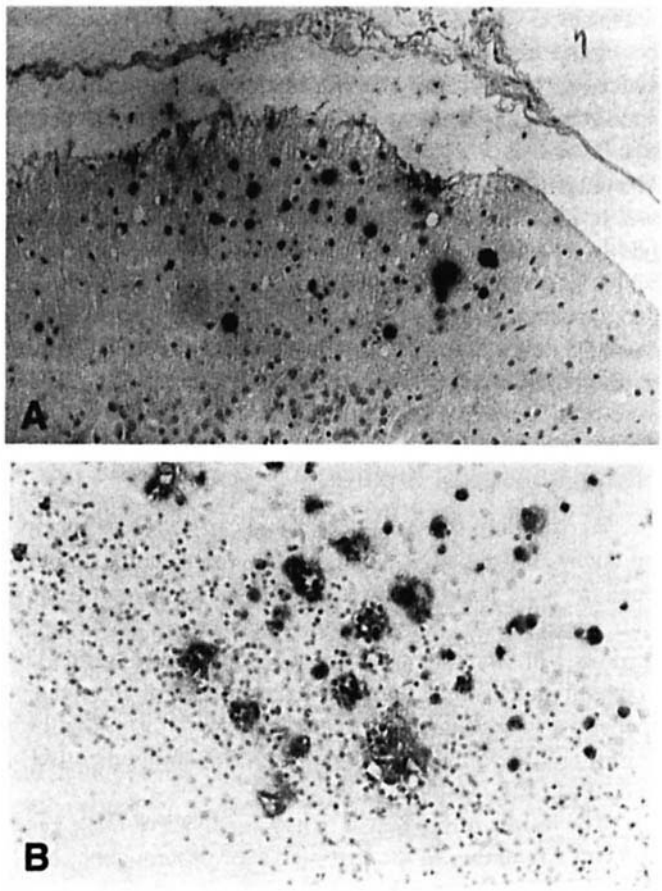

Figure 4. (A and B) Immunocytochemistry for PrP of a section of cerebellum from 47-year-old case shows strong staining PrP positive plaques (×46; 150) and at higher magnification, several multicentric plaques with perivacuolar deposits, ×250. Note the difference in the distribution of PrP-positive plaques as compared with patient EB who had received blood (Fig. 5).

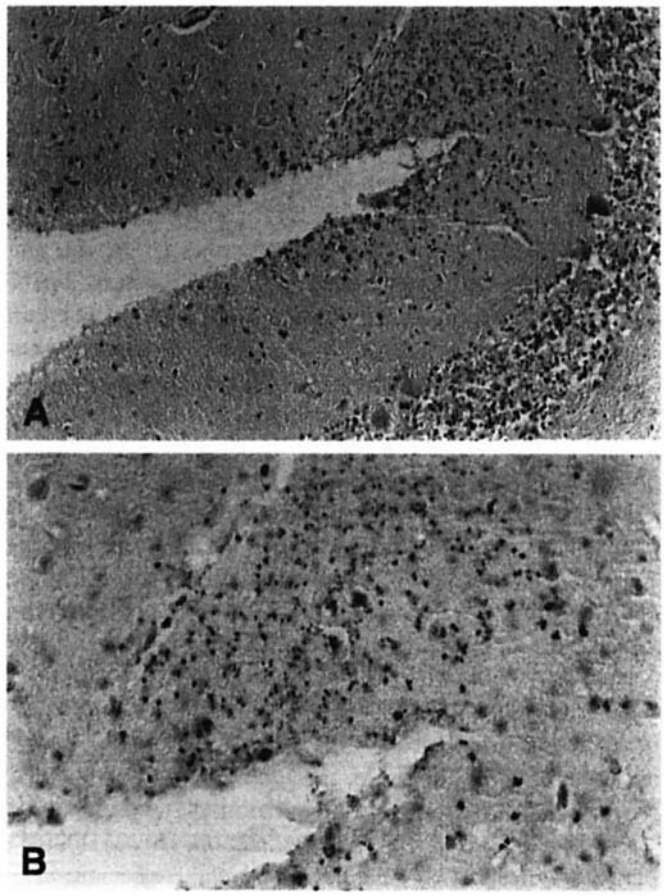

Figure 5. (A and B) Immunocytochemistry for PrP of a section of cerebellum from 59-year-old case shows strong-staining PrP-positive plaques, (×46; 150) and higher magnification shows numerous small multicentric plaques, ×250.

in the monolayer of the cerebellum suggested deposition around small neurons. Differences were very obvious in the size and distribution of PrP plaques in the cerebellum of recipients of pituitary-derived hGH CJD cases when compared with young sporadic CJD patients infected with the BSE agent. The exception was one patient, E.B., who had received blood. The PrP plaques were much smaller in size, as seen in hGH cases (Fig. 5, A and B). This identical size and distribution of PrP plaques in brains of hGH-treated and blood-recipient cases suggests that the blood transfusion case was infected through infected blood.

Following its identification in the 1920s, CJD was recognized as predominantly affecting the middle-aged and elderly. With a few exceptions, where CJD is seen in underage patients, they are "iatrogenic cases." None of the patients included in the study had a potential iatrogenic exposure to CJD through neurosurgery or human pituitary-derived hormone. However, one patient had had a blood transfusion (28).

In the atypical cases, the clinical symptoms and neuropathological features were markedly different from those seen in typical sporadic CJD. The unusual features included the absence of the EEG changes seen in "classical" CJD. Based on conventional and accepted diagnostic criteria for CJD (27), none of these cases would have been considered as even "probable" cases of CJD on clinical grounds. Initially, they had been identified as CJD cases because their brains were examined by touch "impression" and negative staining technique, which demonstrated the presence of tubulofilamentous particles and SAF in their brains and which confirmed the diagnosis (41, 47). This technique helped to identify and confirm the diagnosis. In sporadic CJD cases, confluent spongiform changes in the cerebellum are very rare. However, in all six cases examined in this study, there was extensive vacuolation seen in the cerebellum. The neuropathological lesions in each of these cases were virtually identical and indistinguishable from those reported previously (42). To strengthen their hypothesis of a new strain of the CJD agent, Will *et al.* (42) compared brains from a 27-year-old patient from Poland and from a 16-year-old patient from England who had died of CJD in 1980. They also reviewed 14 cases of CJD patients under the age of 30, previously reported from outside England. In this review, plaques are described in only one case, and in that case, the possible diagnosis of GSSS was considered. In addition, they examined 175 sporadic cases of people who had died since the start of the BSE epidemic, all from England, and they did not observe this unusual feature in a single one.

Since they did not see PrP plaques in recent, older patients, nor had they seen them in those young patients dying prior to the BSE epidemic, they considered that this new variant of CJD affected only the young.

Another main difference between the "old classic" and "new" strain CJD is that the classic disease starts with "dementia" without PrP plaques present, while the "new" starts with "balancing," and difficulty in walking. All of these patients have extensive vacuolation in the cerebellum and PrP plaques in their brains. These are the three major differences that distinguish sporadic cases from those caused by the "BSE" strain of the agent. Based on the clinical and histopathological differences, the six cases over the age of 40 described here were all infected with the "BSE" strain of the agent. Age of patients should not be a criterion to substantiate the claim that older people are not being infected with this strain of the agent. The estimate that only 46 persons died from this strain as of September 1999 may be a gross misrepresentation of the true numbers, which could be substantially higher.

A detailed study of the history of scrapie has revealed that this so-called "nvCJD" is one of the old, rare strains of scrapie in sheep the "trembling type" (1, 2, 28). The BSE strain when transmitted to mice, sheep, and mink shows very little difference in incubation period between the inoculated and oral routes. However, an important difference observed between strains was the susceptibility to UV irradiation. The susceptibility of the "drowsy" strain was reduced by 99% by levels of germicidal UV irradiation that had no effect on "hyper" strains of transmissible mink encephalopathy (TME) agent (4, 48). Given this property, the rare strain of the agent selected by cattle survived the rendering processes and, during recycling cattle to cattle, became the major strain. This rare strain being selected has become the dominant strain to infect humans and other farm and zoo animals. As it is an old strain, the name "new variant strain" is therefore misleading.

CJD and Blood Transfusion

In experimental animals, blood of CJD patients has been shown to be infectious, both during the incubation period and the clinical phase of CJD (49–53). Klein *et al.* (49) and Tateishi (52) independently transmitted the disease from crude suspension made in normal saline of the brain, cornea, and untreated cerebrospinal fluid (CSF) from blood samples and urine from a patient infected with CJD.

Esmonde *et al.* (54) in one epidemiological study in England of 202 definite and probable cases identified 21 CJD patients who had received a blood transfusion and 29 who had donated blood. The mean interval from blood transfusion to the onset of the clinical symptoms of CJD was reported to be 174 months, with a median of 114 and 118 and a range of 2 to 588. The clinical features recorded in the blood transfusion CJD recipients were similar to those observed in the sporadic cases, and therefore, Esmonde *et al.* (54) suggested that blood transfusion is not a major risk factor for CJD. At the same time, the authors also concluded that epidemiological evidence does not exclude the possibility that isolated cases of CJD are caused by the transmission of the causative agent through transfused blood. Results of comparative pathology, combined with immunohistochemical staining, demonstrates the possibility that isolated cases are being caused by the transmission of the agent through transfused blood.

In Conclusion

Since the first appearance of BSE, CJD has been identified in young patients. However, based on the three main distinguishing features described above and on a literature review, it has been revealed that patients of all age groups have died of CJD, but have not been recorded as such; older patients have been disqualified by age. The term "new variant" (nvCJD) was introduced because it was thought to be a new strain. However, realizing that the disease pre-existed, the term "variant" CJD (vCJD) has recently been introduced. A study of Icelandic sheep scrapie and review of the European scientific literature has demonstrated the existence of two strains of scrapie in sheep: Type I, "itchy" and Type II, the ataxic "trembly" type. Cases of the ataxic-cerebellar form of CJD were described 20 to 30 years before BSE appeared. These clinical signs, trembling and ataxia, are similar in BSE, vCJD, kuru, and in sheep inoculated with brain tissues from cows. Since the clinical signs of Type II scrapie in sheep with trembling and ataxia are similar to those seen in BSE and nvCJD, this suggests that Type II is the cause of BSE and vCJD. Many "atypical" cases, with all the unusual leading clinical features such as difficulty in balancing and ataxia, have been reported in patients. Because PrP immunostaining techniques were unavailable in the past, these patients' brains should be re-examined for PrP plaques, along with CJD patients who have received a blood transfusion. The pattern and distribution of PrP plaques should be used as a guide to determine the strain and source of infection and confirm that the name nvCJD is misleading.

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